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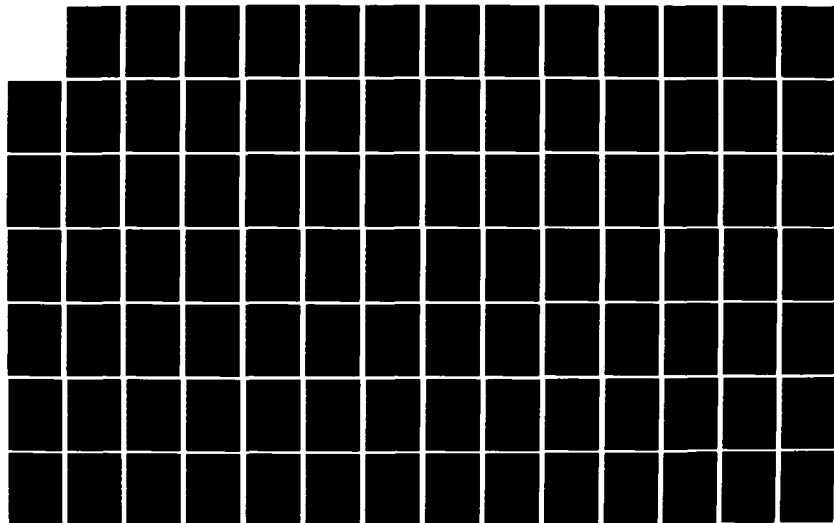
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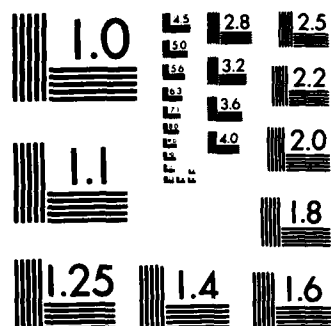
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DYNATECH R/D COMPANY

ASSESSMENT OF WOUND THERAPY SYSTEMS

Final Report
Contract N00014-81-C-0468
Dynatech Technical Report No. 2273

Submitted to:

U.S. Naval Medical Research and Development Command
National Naval Medical Center
Code 45
Bethesda, Maryland 20014

Attention:

Commander Michael Strong, Ph.D.

Prepared By:

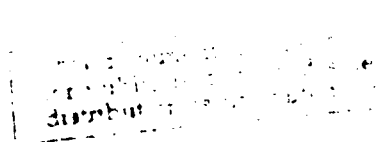
Stanton de Riel
Judith P. Kitchell, Ph.D.



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Submitted:

October 6, 1983



a division of DYNATECH CORPORATION

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Section 1

OVERVIEW

1.1 Introduction

The project goal is the development of a wipe-on burn covering which stores easily, applies readily, adheres for several days, and peels off without trauma. A wipe-on system consisting of a polycaprolactone polymer was developed. This system employed an organic solvent which was unsuited to medical use. The investigation of a more desirable medium for this system is described in Section 5. During the period of April 1982 through March 1983, two water-based film forming systems were investigated. One of these film systems employed gelatin, the other, a synthetic polymer called PViPAA (polyvinylpyrrolidone/allylamine). Preliminary characterization of these systems, including in vivo testing on burns and unburned rats, was reported in our recent Annual Project Report (Dynatech Rpt. No. 2245) and is covered in this report in Sections 2, 3 and 4.

More recently, work has focused only the PViPAA system, because it appears to be quite biocompatible and does not pose the threat of antigenicity, which is a concern in the use of gelatin. In Section 2, this ~~final~~ report covers five areas of study of PViPAA not reported earlier: effects of pH on PViPAA gels, effects of salinity on PViPAA gelation, absorption/resorption of bovine serum albumin on PViPAA gels, effects of some additions to PViPAA gels, and release of antibiotics from PViPAA gels. A new material derived from PViPAA is described in Section 6. Section 7 is a discussion of other medical applications for PViPAA films. In Appendix A are copies of all Dynatech disclosures generated under this contract. An evaluation of a PViPAA covering on an excision wound is given in Appendix B.

1.2 Background

The area of burn wound covering has received significant medical and scientific attention. For standard civilian and conventional

military situations involving a severely burned patient, the current procedure is to immediately--usually within 24 hours and certainly never greater than 48 hours--bring the severely burn-wounded patient to a burn/trauma center. Here the third degree burn wound is excised and a covering applied. Much research and development has been devoted to these post-excision burn wound coverings. These coverings are well-developed, although challenging research problems still exist.

Under military or civilian situations where this modern, efficient treatment system may not be able to function, essentially no satisfactory immediate, emergency medical procedure exists. The only procedure available today is to keep the patient quiet and wrapped in a blanket--often with fatal results. Clearly this cannot be tolerated by a modern military force.

An immediate post-burn treatment could be useful in two situations. In the first, it would be a temporary covering to prevent infection, water loss, and penetration when the patient could not be immediately evacuated, but had to wait for up to 24 to 48 hrs for hospital treatment. In the second, it would also serve as a protective covering, but for a protracted time.

Consider this scenario. A missile attack on a ship at sea leaves a third of the military personnel dead, a third burned to some degree, and the remaining third functional. Due to deployment of helicopters and rescue vessels elsewhere in such an emergency or inability to get to the ship that is hit, there is no possibility of implementing conventional burn wound treatment, i.e., evacuation to a burn wound center for excision and application of post-excision coverings. The question must be addressed in military circles--how are the burn wound patients to be cared for not just during the first 24-48 hours, but perhaps up to 7 days when conventional treatment is not available? Direct questions to leading medical and scientific experts working in the area of burn wounds indicate that there is no treatment procedure suitable for on-ship care during this type of military emergency period.

1.3 Summary of Results with Poly(vinylpyrrolidone/allylamine) Crosslinked Polymer

Co-polymerization of vinylpyrrolidone and allylamine produces a water-soluble polymer (PViPPA) in a molecular weight dependent on the ratio catalyst to starting materials. This type of polymer is readily crosslinked with glutaraldehyde and the crosslinked product is water insoluble. The rate of crosslinking is pH dependent; firmness of the gel develops in less than a minute at basic pH, while at slightly acidic pH solidification is seen within 1 - 5 minutes.

After the crosslinking reagent is added to the polymer, the resulting solution may be spread as a film before gelation occurs. When the crosslinking is done in aqueous solution, the resulting film dries and becomes somewhat brittle. With the addition of glycerol or polyethylene glycol E-400, the film remains strong and pliable.

Pervaporation studies with these films demonstrated that water-vapor transmission, although higher than that of human skin, was substantially reduced from the rate for water loss from open containers. The specific rate of loss through a film of 0.0991 cm thickness was $4.31 \times 10^{-4} \text{ g hr}^{-1}\text{-cm}^{-1}$. The rate was proportioned to the thickness according to the following formula:

$$\text{Rate of loss (g hr}^{-1}\text{cm}^{-2}\text{)} = k(\text{thickness in cm})^{-0.45}$$

In vitro release of antibiotic from PViPAA films was measured. The rate of release was dependent on the solubility of the drug in the leaching media. Sulfadiazine, a relatively insoluble drug at pH 7, was released over a 3-day period. The total amount delivered was 1.25 mg/cm^2 . Penicillin G was released more rapidly; 50% was released in the first hour; the amount released in less than a day was 4.5 mg/cm^2 .

The work on PViPAA is covered in detail in Section 2.

1.4 Summary of Results with a Collagen Based Wipe-On System

A promising wipe-on system based on the deposition of insolubilized polymer films from aqueous protein hydrolysate solutions has been investigated. Experiments have been conducted with two grades of collagen hydrolysate (gelatins) which have been cross-linked in situ from water solution. This work clearly shows that stable insoluble films can be cast from water solution on surfaces of complex curvature. These materials may prove to be useful as "wipe-on" burn dressings because they are easily applied, may be stored at room temperature in water solution, are non-flammable, and non-toxic.

A commercially available gelatin may be dissolved in water and crosslinked with the addition of formaldehyde. The crosslinking reaction is slow enough that the solution may be wiped or brushed onto a surface before the gel solidifies. When glycerol is incorporated into the reaction mixture, the resulting film remains supple for many days.

During exposure of the film to air, most of the water content is lost, but the glycerol remains. Water vapor is transmitted through the "dry" film at a mean rate of $7.0 \times 10^{-4} \text{ g} \cdot \text{hr}^{-1} \cdot \text{cm}^{-1}$.

As a first step toward incorporation of an antibiotic in the film, a model compound was used. Phenolphthalein, an easily identifiable phenolic compound of small molecular weight was mixed with the gelatin before addition of the crosslinking reagent. After the film was formed and equilibrated, it was immersed in distilled water. Periodic samples of water were analyzed for phenolphthalein content. After two days, 15% of the model compound had been released, half of this total was released within the first three hours.

An in vivo study of the adhesive quality of the gelatin film was performed. Rats were the subjects of the experiment. Every rat was anesthetized and an area on each rat's back was shaved and depilated. One half of the rats received second or third degree burns in the bare area.

Fresh gelatin solution, with crosslinking reagent, was prepared and brushed evenly on the bare skin or the burned area. A strip of gauze was placed over the film and a further application of gelatin solution was applied. Two formulations were tested incorporating varying quantities of glycerol plasticizer.

Coverings were removed after 2 or 24 hours. At two hours, the mean adherence of the film on the burned animals was 0.12 - 0.16 lb/in, depending on formulation. Significantly less adherence, 0.05 - 0.06 lb/in, was found for the film on intact skin. At 24 hours, the adherence had increased to 0.18 - 0.27 lb/in for both groups of controls (unburned), but did not increase significantly for the burned animals, 0.16 - 0.24 lb/in, again depending on formulation.

In every case, the gelatin film adhered well and was removable without injury to burned or unburned tissue. Full reporting of this work is given in Section 2.

1.5 Summary of In Vivo Results Using Gelatin and PViPAA Films with Antibiotics

Twenty-four rats were used in these tests. Twelve animals were given burns; twelve were only depilated. Half of each group received gelatin films, the other half received PViPAA film coverings. Within these sub-groups, 3 rats had films with penicillin, and 3 rats had sulfadiazine in their films. The animals were kept under observation, and urine and feces were collected daily for 3 days.

The animal tests provided information about the adhesion of the antibiotic-loaded films and about the prevention of infection and fluid loss. Of the twelve burned animals, two died during the second 24-hour period and one on the third day. No signs of infection were reported in the burned areas and signs of healing were reported in one animal. The films adhered better to burned than to unburned skin, it is postulated that the losses of adherence were primarily due to the abrasion of the rats' backs

against the cage walls and ceiling. The differences in observed adhesion to burned vs. unburned tissue may in part be related to the relative activity of the animals, the burned rats being more subdued, and to hair regrowth on unburned skin. In spite of these difficulties, it is clear that wound protection for several days is feasible under non-abrasive conditions.

Radiolabelled antibiotics were used, and excreta were examined for these labels. It was plain from the urine and feces analysis that portions of the films had either been ingested or, in spite of precautions, directly mixed with the excrement. There was a higher correlation between percent loss of film and amount of label in the urine and feces than between any other parameters. The amount of label recovered was in all cases less than 10% of the total applied. Further analysis of the excretion data was not possible.

Section 2

DEVELOPMENT OF COPOLYMERS OF VINYLPIRROLIDONE AND ALLYLAMINE AS WOUND COVERINGS

2.1 Overview on Polyvinylpyrrolidone

Polyvinylpyrrolidone (PVP) is a water-soluble polymer which is useful in a very wide range of applications -- most notably as a blood plasma extender. PVP was used extensively during World War II for this purpose. In the blood it apparently sequesters small molecules, for instance, toxins, or administered drug compounds.* The well-known antibiotic Betadine™ is a PVP with iodine molecules intercalated within polymer coils.

2.2 Preparation of the Copolymers

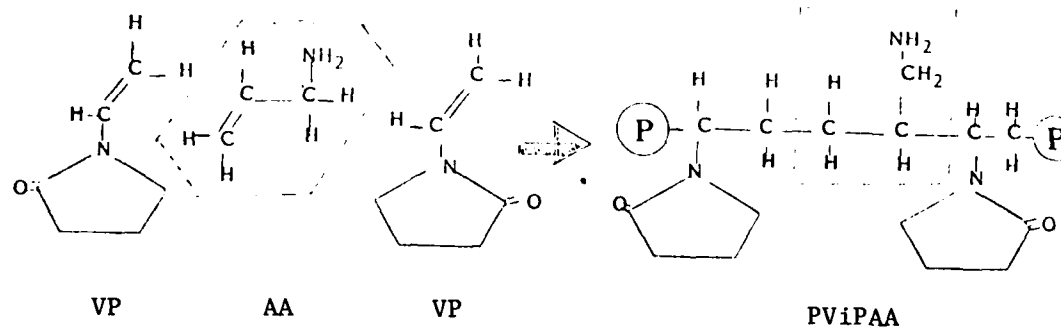
We reasoned that because PVP has a respectable medical history and because it has the advantage of water solubility, it would be an excellent candidate for a burn covering. The disadvantage of PVP is that it is not easily crosslinked just prior to wound application, because it has no readily reactive groups. We chose to synthesize a copolymer of vinylpyrrolidone (VP) and a small percentage of allylamine (AA). This product (PViPAA) has free amines for use in a later crosslinking step. This reaction is shown in Figure 2.1.

In our first preparations of PViPAA, using hydrogen peroxide as a catalyst, we found that the amine in the polymerizing solution caused rapid decomposition of H_2O_2 . Several other peroxides, hydroperoxides, peresters, azo compounds, and persulfates were tried as initiators. These preparations were made in alcohol as not all initiators were water soluble. Hydroperoxides and azo-bis(isobutyronitrile) were slightly better than the other initiators. t-Butylperbenzoate (TBPB) was chosen for subsequent work.

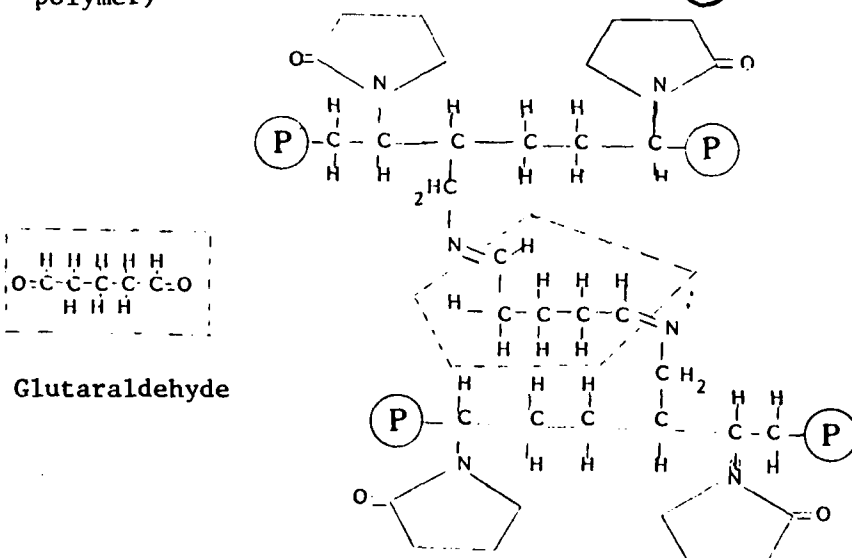
* General Aniline and Film Corporation NY (1951). PVP-Polyvinylpyrrolidone: Preparation, Properties and Applications in the Blood Field and Other Branches of Medicine.

Figure 2.1

Copolymerization of Vinylpyrrolidone and Allylamine



- A. Copolymerization of Vinylpyrrolidone [ViP] and Allylamine [AA] to Form Poly (Vinylpyrrolidone) [PViPAA] ((P) represents the extended polymer)



- B. Crosslinking Product -- Two Strands of Poly(Vinylpyrrolidone) [PViPAA] With One Glutaraldehyde

Small scale polymerizations using 40% VP, 0 - 40% (by volume) allylamine, 0.1% TBPB, and 40% VP, 1.2% allylamine, 0.03 - 3% TBPB were run. All were incubated at 60°C overnight. Use of 0.4% amine decreased the molecular weight of the product dramatically (~ 70%) from that expected from a preparation under similar conditions, but without any allylamine. An initiator level of 1% gave the most viscous solutions. These results indicate that allylamine may induce some transfer-termination, but that increased initiator loading may partially counteract this.

Based on this work, two larger batches were prepared with the proportions of components as shown below in Table 2.1.

Table 2.1
RECIPES FOR TWO BATCHES OF PVIPAA

BATCH	VOLUME RATIOS			
	SOLVENT ALCOHOL	VINYLPYRROLIDONE	ALLYLAMINE	TBPB
A	6	4	0.12	0.3
B	6	4	0.12	0.03

Reaction conditions: 80°C overnight under N₂.

Gel permeation chromatography (GPC) on samples from both batches indicated that three individual polymeric fractions were present, although more markedly in A. The M_w 's calculated for these are shown in Table 2.2. Peak No. 2 has opposite refractive polarity versus DMF from peak Nos. 1 and 3.

2.3 Optimizing Initiator Concentrations

Because previous experiments has indicated that the molecular weight of PVIPPA was dependent on solvent composition, this factor and also

Table 2.2

GEL PERMEATION CHROMATOGRAPHY ON TWO PVIPAA SAMPLES

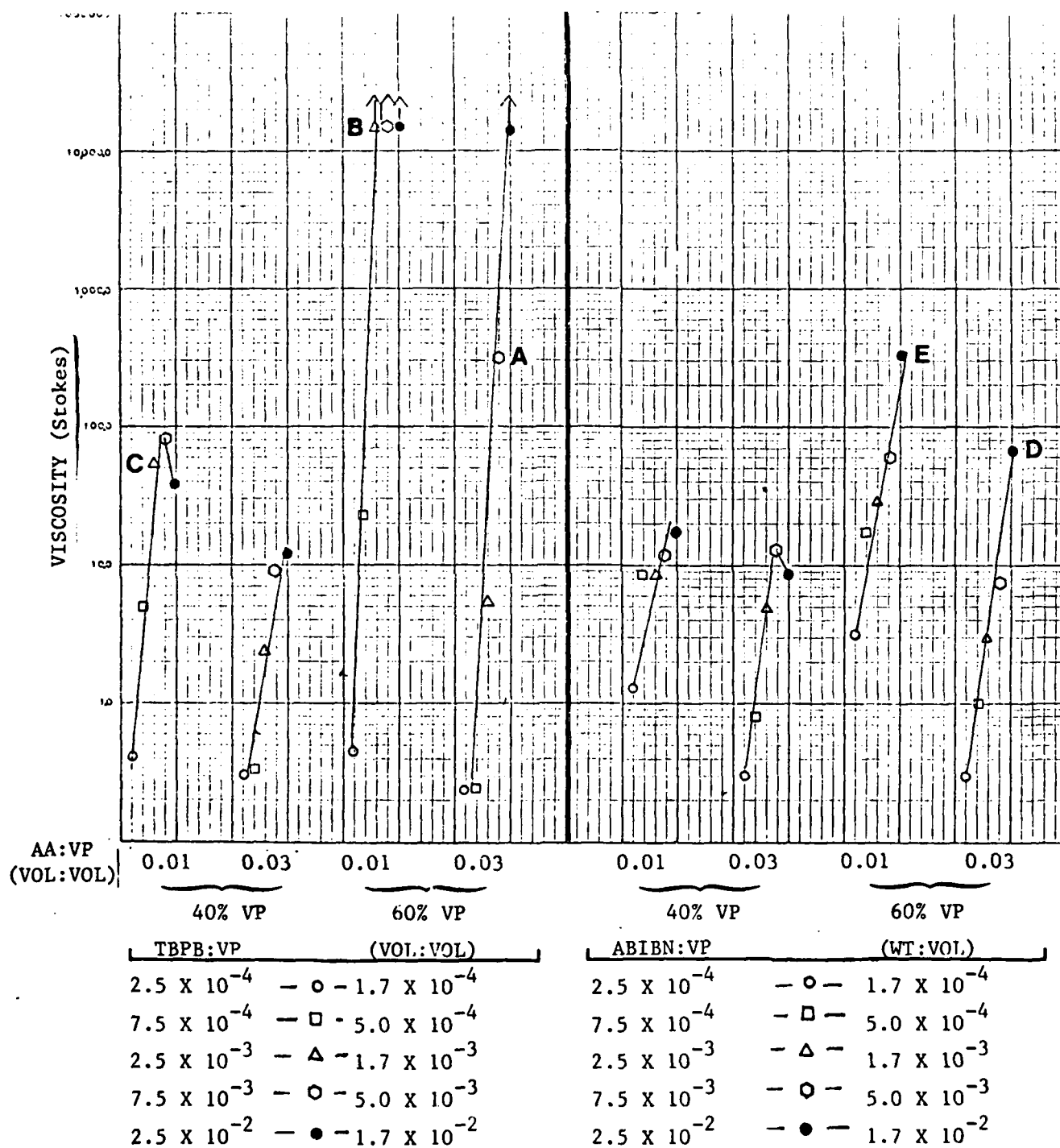
BATCH	COLOR	M _w , (% of Total GPC Area)		
		PEAK 1	PEAK 2	PEAK 3
A	Medium Yellow	9300(83)	Obscured	900(17)
B	Light Yellow	27700(73)	2700(22)	1500(5)

variations in quantity of initiator were investigated for two monomer mixes. Thus, at proportions of 4:6 and 6:4, reagent alcohol:vinylpyrrolidone (VP) (vol:vol), and at monomer mixes of 0.01 and 0.03:1 allylamine:VP (vol:vol), different quantities of initiator were employed using the two initiators, *t*-butylperbenzoate (TBPB) or N,N'-azobisisobutyronitrile (ABIBN). Each reaction mixture, on a 10 ml scale, was prepared by sealing nitrogen-purged ingredients in 3 dram vials. The vials were kept, without shaking, at 80°C for 40 hr. Product viscosities after 16 hrs approached the final ones; thus, we are certain that the observed results demonstrate essential differences in initiator action. The experimental grid and resulting product viscosities are shown in Figure 2.2. It may be seen that TBPB produced much higher weight material than ABIBN; however, it was also more sensitive to the pressure of AA.

Selected samples from this study were examined for gelation ability. Those reaction mixtures labeled A-E in Figure 2.2 were separately precipitated into diethyl ether, filtered and dried. The resulting polymers were made up as 5, 10, 15, and 20% aqueous solutions at pH 9.3 - 10.3. Glutaraldehyde concentration was held constant at 0.17%. The gelation behaviors at 10 minutes are shown in Table 2.3.

Figure 2.2

VISCOSITIES OF PViPAAs PREPARED UNDER VARIOUS REACTION CONDITIONS



Key: AA = Allylamine
 VP = Vinyl Pyrrolidone
 TBPB = *t*-butylperbenzoate
 AIBN = *N,N'*-azobisisobutyronitrile

Reaction conditions are described in the text.

Table 2.3

GELATION OF SELECTED PVIPAA SAMPLES†

% PVIPAA	SAMPLE*				
	A	B	C	D	E
5	None	None	None	None	None
10	Medium gel	Trifle stringy	None	None	None
15	Firm gel	Medium gel	Delayed soft gel	None	None
20	Very firm gel	Firm gel	Soft gel	None	None

* See Figure 2.2 for sample designations.

† At 10 minutes after mixing.

2.4 Crosslinking Procedures

Glutaraldehyde was used as a crosslinking reagent. The effect of the concentration of polymer on gel formation at a fixed glutaraldehyde concentration (3.6%) was studied using polymers A and B in water. Table 2.4 shows the type of gelation observed and the relative speed of the reactions.

Table 2.4

EFFECT OF POLYMER CONCENTRATION ON
GEL FORMATION WITH 3.6% GLUTARALDEHYDE

% POLYMER (WT/VOL)	SAMPLE	
	A	B
10	None	None
20	None	Moderate
30	None	Rapid, firm
40	Mild	Rapid, firm
50	Moderate	Rapid, firm
60	Rapid, firm	Rapid, firm

Reaction conditions: 23°C, 3 ml samples in glass vial, observation over 5 minutes.

Further work with 20% B at pH 9.2 established that with 1% glutaraldehyde, gelation was immediate; use of 0.4% glutaraldehyde gave set

up in 30 seconds. Addition of 0.2% glutaraldehyde gave a weak gel in several minutes. Using 40% B and 0.16% glutaraldehyde, an immediate, progressively firming gel developed. When the amount of glutaraldehyde was decreased to 0.04%, soft gel was formed.

At pH 6.6, gelation was slower than at pH 9, but the final firmness was unaffected. At pH 2, no gelation took place, but as pH was raised with NaHCO_3 , a uniform, foamy gel formed.

2.5 Plasticizers and Surfactants

We have tested glycerol, triacetin, di(2-methoxy ethyl) phthalate, polyglycol E400, 1,3-butyleneglycol, and polyvinylalcohol as plasticizers with PVIPPA.

In each case, 7% plasticizer (vol/vol final) was used with 20% polymer and 0.17% glutaraldehyde (pH 5.9). The solution was cast onto a glass plate as a 0.020 in. film. At this pH, gelation requires ≥ 10 minutes on all films or in bulk.

Of these plasticizers, the phthalate and triacetin blush out and are unsuitable. Polyvinylalcohol forms a hard, brittle film quite resistant to solubilizing by H_2O ; the butyleneglycol forms a slightly tacky gel, which eventually loses plasticity; glycerol and the polyglycol plasticize effectively and do not dry out; whereas an unplasticized gel film dries to brittleness.

Biocompatibility consideration of polyglycol vs. glycerol favors the latter as a plasticizer for wound-covering applications. The optimal proportion of glycerol to be used was determined in a two-phase testing procedure. First, a broad survey of possible values was tested. The results, shown in Table 2.5, narrow the focus to between 35 and 45% glycerol/polymer. In Table 2.6, the optimal ratio is shown as 38%. This film is pliable and relatively tough.

Table 2.5

PRELIMINARY INVESTIGATION OF GLYCEROL AS A PLASTICIZER

% GLYCEROL (TOTAL)	% GLYCEROL/ POLYMER	FILM DRIED OVERNIGHT, SCRAPED- OFF GLASS PLATE WITH RAZOR
3	15	Crumbles
5	25	Cracks extensively
7	35	A few cracks
9	45	No cracks, easy to manipulate
12	60	Buckles and stretches
15	75	Far too limp to manipulate

20% polymer, 0.1% sodium dodecylsulfate, pH 6.0

Table 2.6

REDUCED RANGE INVESTIGATION

% GLYCEROL/ POLYMER	FILMS DRIED 1 DAY, REMOVED BY RAZOR
38	Non-creasable, pliable
42	Limp
46	More Limp
50	Very limp and easily Stretched

20% polymer, 0.1% sodium dodecylsulfate, pH 6.0

It is of most interest to study the polymer gel as a film. Spreadability depends not only on the nature of the gel, but also on the interaction of the gel with the casting surface. We found that the addition of sodium dodecyl sulfate (SDS) in small amounts (i.e., 0.05%) aids in adhesion of the gel to glass, foil or cellophane.

2.6 Preparation and Pervaporation Measurements on PViPAA Films

Film Preparation

Films of various thicknesses for pervaporation studies were prepared as follows:

Polymer Solution "A"

11.71 g PViPAA 016442-1
14.5 g methanol
9.20 ml 50% wt/vol (aqueous) glycerol stock solution
0.035 g SDS
23 drops 1N HCl (q.s. to bring to pH 6.00)

Gelation Inducer "B"

3.5% (wt/vol) (aqueous) EM-grade glutaraldehyde

Polymer solution "A" was prepared by sequentially adding and dissolving the listed ingredients (Note: glycerol is "cut" with water to make its manipulation easier), then portioned into individual mixing vials. To each vial, gelation inducer "B" at the rate of 0.8 ml "B"/5g of "A" was added, the mixture quickly mixed with a wooden applicator stick, and the contents of the vial immediately poured into a glass Petri dish. The dish was vibrated gently to assure even distribution of the gel medium, then allowed to gel undisturbed. Increasing gelation took place gradually over a period from one to about 15 minutes after mixing. Films were allowed to air dry (of water and methanol) overnight at 20°C, stripped from their support surfaces, cut by scissors to size, and clamped into position on Payne Permeability Cups (Fisher).

Net weight (immediately after casting) and dry weights (before removal from supports) on films 016731-1, -2, and -3 are shown in Table 2.7,

Table 2.7

PERVAPORATION MEASUREMENTS ON PVIPAA FILMS

Film	016731-1	016731-2	016731-3
Wet weight, net (g)	3.60	5.15	12.47
Dry Weight, net (g)	1.64	2.41	5.96
% dry/wet	46	47	48
Film Area (cm ²)	56	45	65
Calculated Dry Film Weight/Area (g/cm ²)	0.030	0.055	0.092
Ave. Hydrated Film Thickness (cm)	0.0330	0.0635	0.0991

along with the calculated dry film weight/area, and in-use average hydrated film thicknesses (measured at 6 points on the exposed film area (9.62 cm²) during pervaporation testing).

The weight loss (due to pervaporation) of the cups was measured daily. After daily measurement, the films were unclamped, lifted, and resealed to relieve inpouching which otherwise occurred due to much greater permeability of the films to water vapor than to air. More distilled water was added if necessary at this time to maintain water level in the cup near 10 ml. Films were then resealed, reweighed, and replaced in the measurement chamber.

The measurement chamber was a 200 ft³ hot room, maintained at 37°C and low relative humidity, with passive air circulation around a perforated metal shelf on which the cups rested. The rates of loss are shown in Table 2.8 and shown graphically in Figure 2.3. Rates of loss for dishes with no film and dishes covered with a Kimwipe® are shown for a comparison.

Table 2.8

WATER VAPOR TRANSMISSION MEASUREMENTS

A. Water Loss From Film-Covered Cups

TIME (HRS) BETWEEN MEASUREMENTS	RAW LOSSES (g)	RATE LOSS $\cdot 10^3$ (g hr ⁻¹ cm ²)	SPECIFIC RATE LOSS $\cdot 10^4$ (g hr ⁻¹ cm ⁻¹)	RAW LOSSES (g)	RATE LOSS $\cdot 10^3$ (g hr ⁻¹ cm ²)	SPECIFIC RATE LOSS $\cdot 10^4$ (g hr ⁻¹ cm ⁻¹)	RAW LOSSES (g)	RATE LOSS $\cdot 10^3$ (g hr ⁻¹ cm ²)	SPECIFIC RATE LOSS $\cdot 10^4$ (g hr ⁻¹ cm ⁻²)
~ 3	0.3063	13.3	4.39	0.3337	11.2	7.11	0.0985	3.53	3.50
24	3.4652	15.0	4.95	2.8280	14.0	8.89	1.6429	8.13	8.05
23.8	2.8435	12.4	4.49	1.8742	8.86	5.62	1.5210	7.19	7.12
22	2.8796	13.6	4.49	1.8742	8.86	5.72	1.5210	7.19	7.12
23.7	2.8730	12.6	4.16	2.1470	9.42	5.98	1.8647	8.18	8.10
23.9	3.0547	13.4	4.43	2.3186	10.1	6.43	2.0798	9.08	9.0
Ave./day	3.0314	13.4	4.42	2.2929	10.4	6.59	1.8071	7.48	7.41
Ave./day after 1 day	2.9446	13.4	4.31	2.1359	9.7	6.13	1.8399	8.1	8.07
TOTAL LOSS	18.50			14.09			10.94		

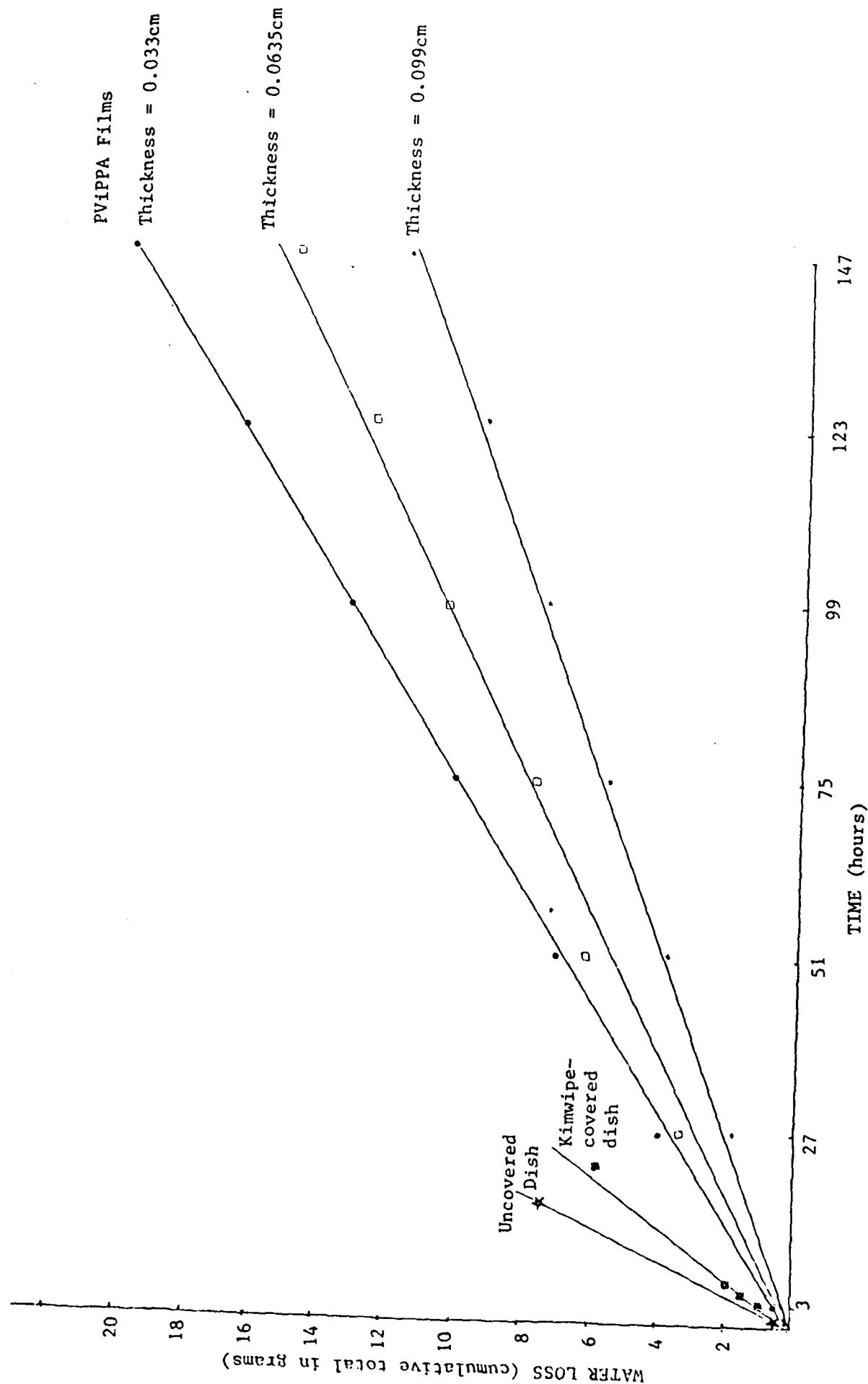
B. Water Loss from Paper-Covered and Open Cups

PAPER COVERED		OPEN	
TIME (hr)	WATER LOSS (g)	TIME (hr)	WATER LOSS (g)
3.38	.9116	1.08	.5083
1.9	.5225	16.2 hr	6.3885
1.03	.2920		
16.2	3.7445		

Conditions: Air exposed at 37°C.

Figure 2.3

Cumulative Water Losses from Covered and Uncovered Dishes



2.7 Effects of pH on PViPAA Gels

2.7.1 Formation of Gels

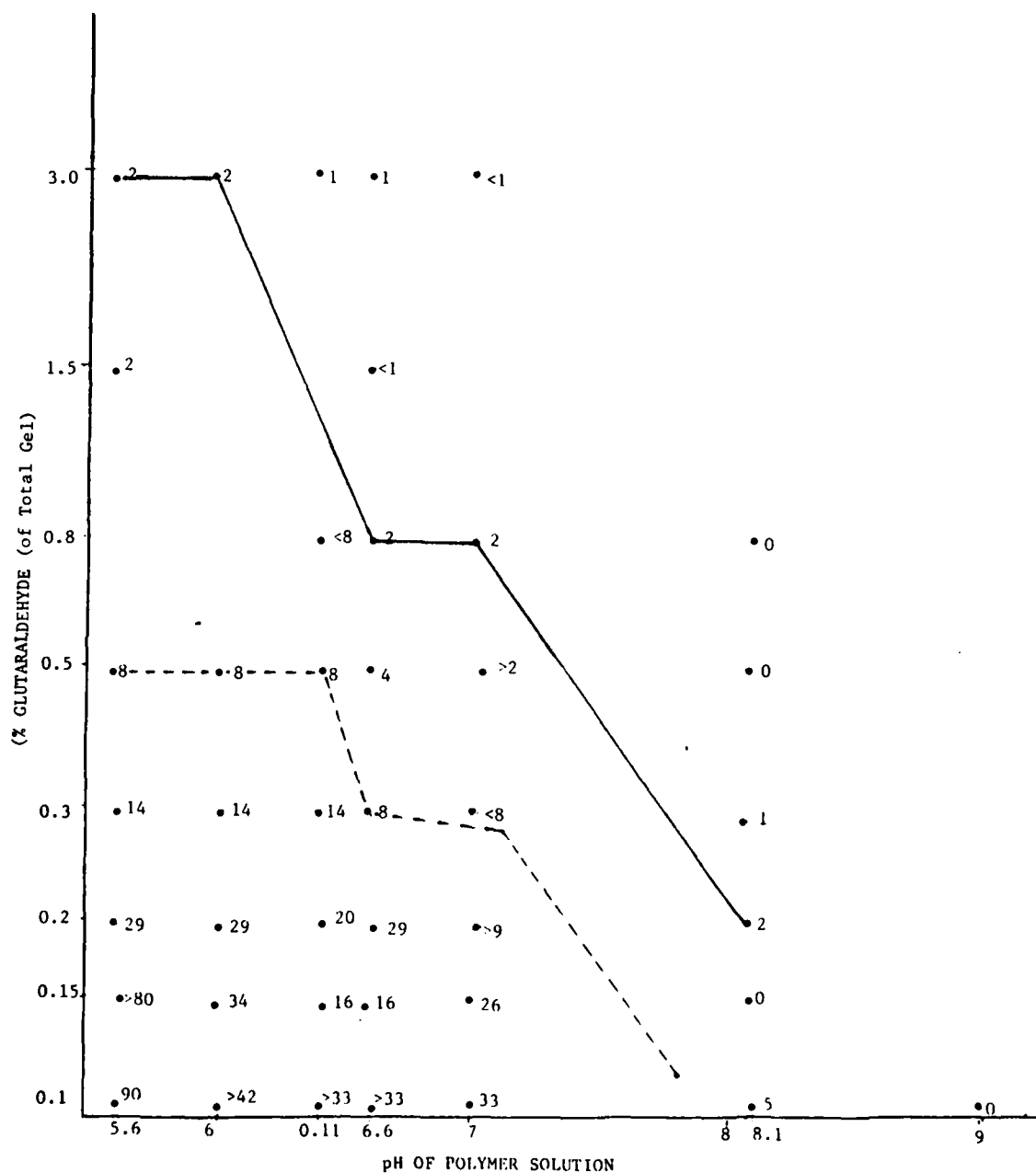
The pH at which the PViPAA-glutaraldehyde crosslinking reaction takes place affects the speed of gelation: higher pHs are assumed to favor unchanged amine groups, which then may attack the aldehydes. A PViPAA solution, which is buffered by its amines, may be adjusted with HCl/NaOH to a desired pH; alternatively, PViPAA may be pH-adjusted in a polar solvent before precipitation into diethyl ether, yielding a custom-adjusted dry product. The gelation speed may be graphically seen in Figure 2.4, in which a 20% PViPAA/H₂O solution is gelled at a range of % glutaraldehyde and pHs (the glutaraldehyde, electron-microscopy grade, is unbuffered and approximately neutral). The times to development of various firmnesses of gel are shown: stringiness, in which the solution starts to form a very thin string, rather than beading, as a probe (2 mm diameter cylindrical wooden stick) is withdrawn above the surface, soft gel, in which the gel just starts to adhere to the probe as a very soft, deformable glob, and medium and firm gels.

2.7.2 Stability of Gels to pH

The stability of PViPAA/glutaraldehyde gels in water at various pHs at room temperature was gravimetrically and spectrophotometrically assayed. A solution of 0.75 g PViPAA + 0.035 g glutaraldehyde in 3.8 g H₂O, was cast and dried at 50°C. The dry gel, weight 0.74 g (loss probably due to residual solvent in the PViPAA), is leached 3X (200 ml water, 1 hr), yielding leachates with UV-250 nm absorbances (vs. H₂O) as in Table 2.9. Thus, approximately 0.25 g PViPAA ($A_{250} = .72 \text{ ml} \cdot \text{mg}^{-1}$) was leached out. The wet gel, comprising approximately 0.49 g dry-PViPAA equivalent, was divided into halves and one portion treated in 150 ml distilled water, to which acid (HCl) was added to stepwise alter pH, the other portion similarly treated with base (NaOH). A_{250} was measured at the end of each leach period (averaging 28 mins duration, except for the final treatment of 16 hrs). The results, in Table 2.10, indicate that at worst, 4.2 and 5.6% of the PViPAA

Figure 2.4

Gelation Times at Varying pH and Glutaraldehyde Concentrations



Legend: The figure at each point is the number of minutes required to reach the soft-gel stage. The heavy line delineates the conditions at which gel time is two minutes; the dashed line delineates conditions for 8 minute gelation.

Table 2.9

NEUTRAL-pH LEACHING OF GEL

LEACHATE NUMBER	A ₂₅₀	PV1PAA (mg/ml)
1	.770	1.07
2	.112	0.16
3	.015	0.02

Table 2.10

ACID/BASE LEACHING OF GELS

Leach with:

ACID (HCl)		BASE (NaOH)	
pH	A ₂₅₀	pH	A ₂₅₀
4.8	.005	8.6	-.005
3.9	.026	9.7	.025
3.1	.038	10.4	.037
2.5	.039	11.2	.040
1.5	.040	11.9	.048
0.9	.038	12.5	.062
0.8	.050	13.0	.072
0.63	.048	13.2	.045

in H₂O, and dried, yielding 0.27 g dried gel each. Thus losses during the pH treatment appear to have been minimal, and these gels may be useful over a wide pH range.

Some alteration of the base-treated gel appears visually to have taken place: the final product is a dark tan, translucent solid as opposed to the transparent yellow of the untreated, and acid-treated, dried gels. It is unknown whether this effect is permanent and if it substantially changes any gel properties.

2.8 Effect of Salinity on PViPAA Gelation

Samples of 20% PViPAA/H₂O were loaded with NaCl to the following levels of NaCl:PViPAA solution: 0, 0.5, 1.0, 3.0, 8.9, and 14.0%. Glutaraldehyde was added, to 0.3% of wet weight; final % NaCl/total of the most highly loaded gel was 12.2%. Although the 8 and 14% NaCl additions caused a slight precipitation of PViPAA, upon glutaraldehyde addition all samples gelled identically to soft-medium stage in 12 mins.

Thus, these gels demonstrate the ability to tolerate electrolyte loadings and pH changes which would make them suitable as a conductive material applied as a liquid, hardening in a short time to a gel, with further modifications possible by incorporation of plasticizers, wetting agents, or bioactive agents.

2.9 Absorption/Desorption of BSA on PViPAA Gel

The ability of pre-formed, leached PViPAA gels to absorb and desorb bovine serum albumin (BSA), as a model protein, was studied. As might be expected from the amidic composition of the PViPAA, BSA is strongly absorbed, as shown in Table 2.11. Each datum here is from triplicate samples of 10 g wet weight (0.39 g dry weight) gel in 200 ml BSA solution. BSA was assayed photometrically at 273 nm. Thus, BSA is absorbed to a maximum level of ~70 mg/10 g wet gel, or 0.18 g BSA/g-dry gel.

Table 2.11

ABSORPTION OF BSA ON GEL

	TRIPLICATE SAMPLES			
	#1 - #3	#4 - #6	#7 - #9	#10 - #12
Equilibrium Solution BSA (mg/ml) $\pm \sigma$	0.11 \pm .01	0.37 \pm .03	1.53 \pm .05	4.46 \pm .03
BSA absorbed on gel (mg) $\pm \sigma$	15.4 \pm 1.2	63.2 \pm 7.2	79.3 \pm 8.2	65.2 \pm 10.7

This BSA is desorbed partially in the presence of 0.3% SDS, and completely at 1% SDS. No desorption occurs with Tween-20 (a non-ionic surfactant) at 1%.

2.10 BSA Leaching from PLGA Matrix

BSA (Pentex Bovine Serum Albumin, Fraction V, Miles Labs) was used as a model protein-type material. To slow the release of this material, and protect it from glutaraldehyde crosslinking, it was incorporated as a suspension in a film of poly(lactic-co-glycolic acid) [PLGA] (Dynatech #B017742-2A, a once-precipitated, not fractionated, 90:10 L:G ratio, $M_w \approx 10,000$ polymer).

Two experiments were performed: a preliminary, in which the BSA release rates upon leaching of films of various %BSA loadings were measured, and a second, in which %BSA loading was kept constant, and BSA release observed as a function of film thickness, from both BSA/PLGA films directly into leachate ('control' samples), and from a PVIPAA gel incorporating BSA/PLGA film flakes ('experimental' samples).

In the preliminary experiment, small films with BSA/PLGA levels of 3, 6, 10, 30 and 50% were cast from milled BSA/PLGA/ MeCl_2 (methylene chloride) (~30% solids) slurries. These proved too viscous for efficient milling, delivery onto the casting plate, or drawing with a Boston-Bradley blade to wet thickness 0.016"; thus, % of BSA released in leaching was not ascertainable. However, the BSA release was followed over time, relative to that at 1 hr (assumed completion). At 20 minutes, these %-of-ultimate-release data ranged from 18 to 80%. Based on this, a low %BSA/PLGA film composition was selected for further work, since any BSA released by such an early time would be lost to crosslinking by residual glutaraldehyde.

In the second experiment, 0.90 g BSA, 15.30 g PLGA, and 35.68 g MeCl_2 were ball-milled overnight with 4-mm glass beads. This reduced the BSA to the desired slowly-settling pulverized state; films of wet thickness 0.004, 0.009, 0.026, and 0.069 in. were drawn on a glass plate, passively

dried in still air 20 minutes, in a cool draft for 3½ hours, then peeled from the glass and vacuum dried at ≤ 4 mm Hg for 18 hrs. The final dried films' areas, weights, and thicknesses were measured (Table 2.12), and portions of films sliced into approximately 5-mm squares. Sufficient quantities of sliced film to contain ≥ 10 mg BSA (where possible) were portioned out, making two non-gel-incorporated 'control' and three gel-incorporated 'experimental' samples for each film thickness, except for the thinnest film, which sufficed for only one sample each of control and experimental. 'Experimental' samples were then one-by-one treated as follows: each was suspended in 30 g of a 20% PViPAA solution in H₂O, 0.25 ml of 2.5% wt/vol E.M. grade glutaraldehyde added, and the lot mixed by vortexing 10 secs. The pre-gel material was poured and transferred by spatula to 250 ml Erlenmeyer flasks, so that all the BSA/PLGA casting squares were delivered to the gel-casting. Gels set in ~4 mins after mixing.

The experimental gels, which now had set for periods of 6 to 30 mins, and the control samples (inserted into flasks as the dry, chopped casting squares) were set to leach with 50 ml phosphate/hydroxide buffer each at 37°C.

Aliquot samples of 100 µl were periodically removed from the bulk of the leachates and analyzed for protein by the Bradford* (Coomassie Brilliant Blue G-250 binding) method. Values so obtained were compared to the known input BSA contents of the samples to obtain values for BSA leached as a % of theoretical input, and the results graphed in Figure 2.5.

Only the 'control' samples (no PViPAA gel incorporation) are shown in Figure 2.5, because only they exhibit any significant BSA release. The 'experimental' samples exhibit a possible absorbance for the thickest film, at best, 0.01 unit, corresponding to 0.018 mg BSA/ml leachate, or 0.9 mg in the leachate. Additionally, a small amount of BSA, possibly up to 4 mg, might be adsorbed on the PViPAA. This total of possibly up to 5 mg BSA, out of 27.5 mg input, i.e., possibly up to 18% leached, contrasts with the 63% leached achieved by the comparable control samples, indicating gross

* Bradford, M.M. 1976. Anal. Biochem. 72: 248.

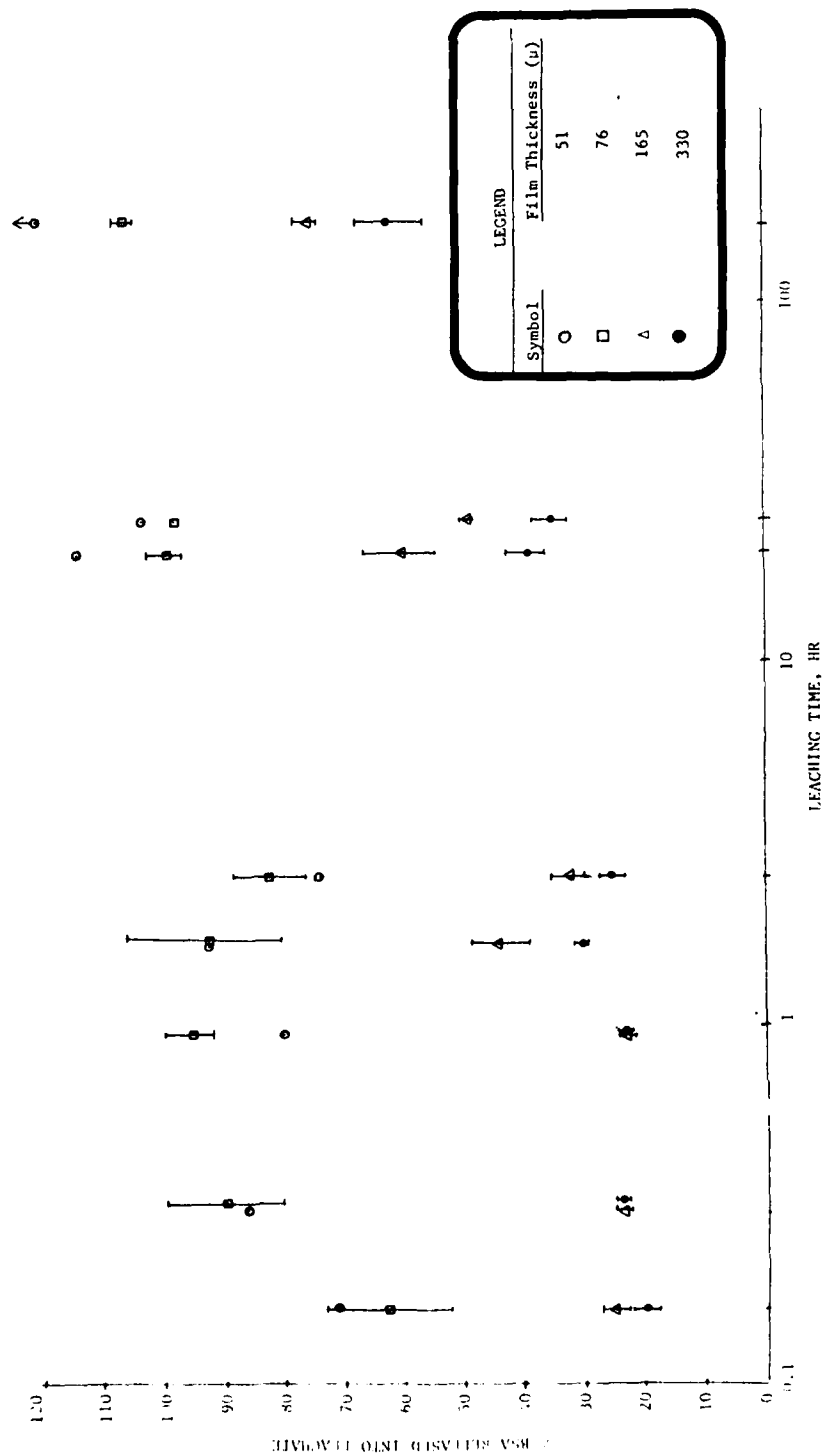
Table 2.12

CASTING AND LEACHING OF BSA/PLGA FORMULATIONS

BSA/PLGA FILM CASTINGS									
Film B018000-	A	B			C			D	
Wet-Cast Thickness (in)	0.004	0.009			0.026			0.069	
Dry Weights (g)	0.280	1.065			2.239			5.585	
Area (cm ²)	124	210			126			128	
Thickness (ave)(μ)	51	76			165			330	
Calculated Density, ρ (g/cc)	0.45	0.67			1.09			1.33	
BSA/PLGA 'EXPERIMENTAL' SAMPLES LEACHED FROM PVIPAA GELS									
Experimental Films #018203-	1	2	3	4	5	6	7	8	9 10
Dry Chopped Film Weights (g)	0.151	.231	.207	.204	.227	.217	.213	.439	.502 .537
BSA/PLGA 'CONTROL' SAMPLES LEACHED DIRECTLY INTO BUFFER									
Control Films #018203-	11	12	13		14	15		16	17
Dry Chopped Film Weights (g)	0.119	.216	.194		.227	.210		.502	.506

Figure 2.5

Release of BSA from PLGA/BSA Castings, Leached in pH 7.0 Buffer 37°C as Assayed
by Coomassie Brilliant Blue G-250 (Bradford) Method



BSA ball-milled in PLGA/MeCl₂ solution, cast and dried, chopped and leached.
Data points are $\bar{x} \pm \sigma$ (2 samples) bars. Points offset for clarity, as necessary.

blockage or crosslinkage of the BSA by glutaraldehyde, even from particles otherwise requiring several days to leach BSA out.

The 'control' samples all release a variable amount of BSA almost immediately. This is hypothesized to be BSA, very near or protruding from the surface of the films. For the two thinner films, this release of ~90% is essentially complete in 20 minutes, while in this period only 25% of the BSA is released from the two thicker films. Both the thicker films then appear to release BSA slowly thereafter, at a rate of around 12%/day for the 165 μ film and 10%/day for the 330 μ film. While this appears to be a very desirably slow release rate, the PLGA does not protect the BSA from glutaraldehyde attack in this formulation.

2.11 Effects of Some Additives to PViPAA/PEG-400 Gels

One method of decreasing the affinity of the protein for the gel is to incorporate a more waxy high molecular weight polymer into the films. Three additives were investigated; a medium weight polyethylene glycol (PEG 3350); a high weight polyethylene glycol (PEG 20,000) and polyvinyl alcohol. In each case, low weight polyethylene glycol (PEG 400) was used as a plasticizer. The results are summarized below.

1) PEG 3350

- a) Considerable opacity is seen at a ratio of 0.2:1 (PEG-3350: PViPAA);
- b) A slight decrease in strain-to-fracture is seen at up to 0.4:1 (PEG-3350:PVIPAA) and a great decrease in strain-to-fracture is seen at \geq 0.6:1 productions.
- c) The film remained pliable.
- d) The bovine serum albumin (BSA) solubility is 10 mg/ml, at between 20 and 30% solutions of PEG-3350 in water.

2) PEG-20M

- a) The films were not pliable.
- b) The films caused great opacity at low levels.

- c) The BSA solubility is 10 mg/ml at between 20 to 40% PEG 20M solutions.

3) Polyvinyl alcohol (PVA)

- a) At 0.031:1 ratio of PVA to PViPAA, the film is less self-adherent, slightly opaque, much less stretchy, but tougher than a film without PVA. Above this PVA level, films become even less stretchy, and more opaque.

2.12 Release of Sulfadiazine and Penicillin-G into Buffer from Films

2.12.1 Leaching of Sulfadiazine

The effect of the presence of polyethylene glycol of molecular weight ~3350 (PEG-3350) on the leaching of sulfadiazine from PViPPA/glutaraldehyde/PEG-3350 films into pH 7.0 buffer, was tested as follows. Ten individual films were prepared and cast with formulations as shown in Table 2.13.

Table 2.13

CONTENTS OF SULFADIAZINE-RELEASING FILMS

FILM NUMBERS	10 - 12	13 - 15	16 - 18	19
PViPAA	0.60 g	0.60 g	0.60 g	0.60 g
PEG 3350	0 g	0.30 g	0.60 g	0.60 g
Sulfadiazine	50 mg	50 mg	50 mg	0
H ₂ O	2.40 g	2.10 g	1.80 g	1.80 g
2.5% Glut. Soln.	0.25 ml	0.25 ml	0.25 ml	0.25 ml

These formulations were prepared in tared 3 dram vials; PViPAA and PEG-3350 were dissolved in H₂O, then the sulfadiazine was added and mixed. When ready to cast, the glutaraldehyde solution was injected into each vial. The vial was vortexed three seconds, then the contents were immediately poured into a 250 ml Erlenmeyer flask, allowing as much of the viscous solution to drain as possible. The flasks were tilted and shaken to spread the formulation over the flask bottom, ~38 cm² area; however, the gelled films were somewhat irregular in thickness. The vials were recapped and weighed to

determine the residual, undelivered formulation (%-delivered-into-the-flask varied from 76 to 92%). The gels were observed to set to no-flow hardness in 5, 4, 2-3/4, and 2 min for the four film groups in the formulation table, respectively. After 25 min of setting time, leaching was started by adding 200 ml of leaching medium to each flask, consisting of 0.05 M $\text{KH}_2\text{PO}_4/\text{NaOH}$ pH 7.00 (25°C) buffer (Fisher Scientific). Temperature of incubation was 34°C throughout the experiment; buffer was pre-warmed before use. At two points (5.3 and 29 hrs) the buffer was changed to fresh buffer immediately after sampling, to ensure that the leaching was not limited by saturation of the leachate.

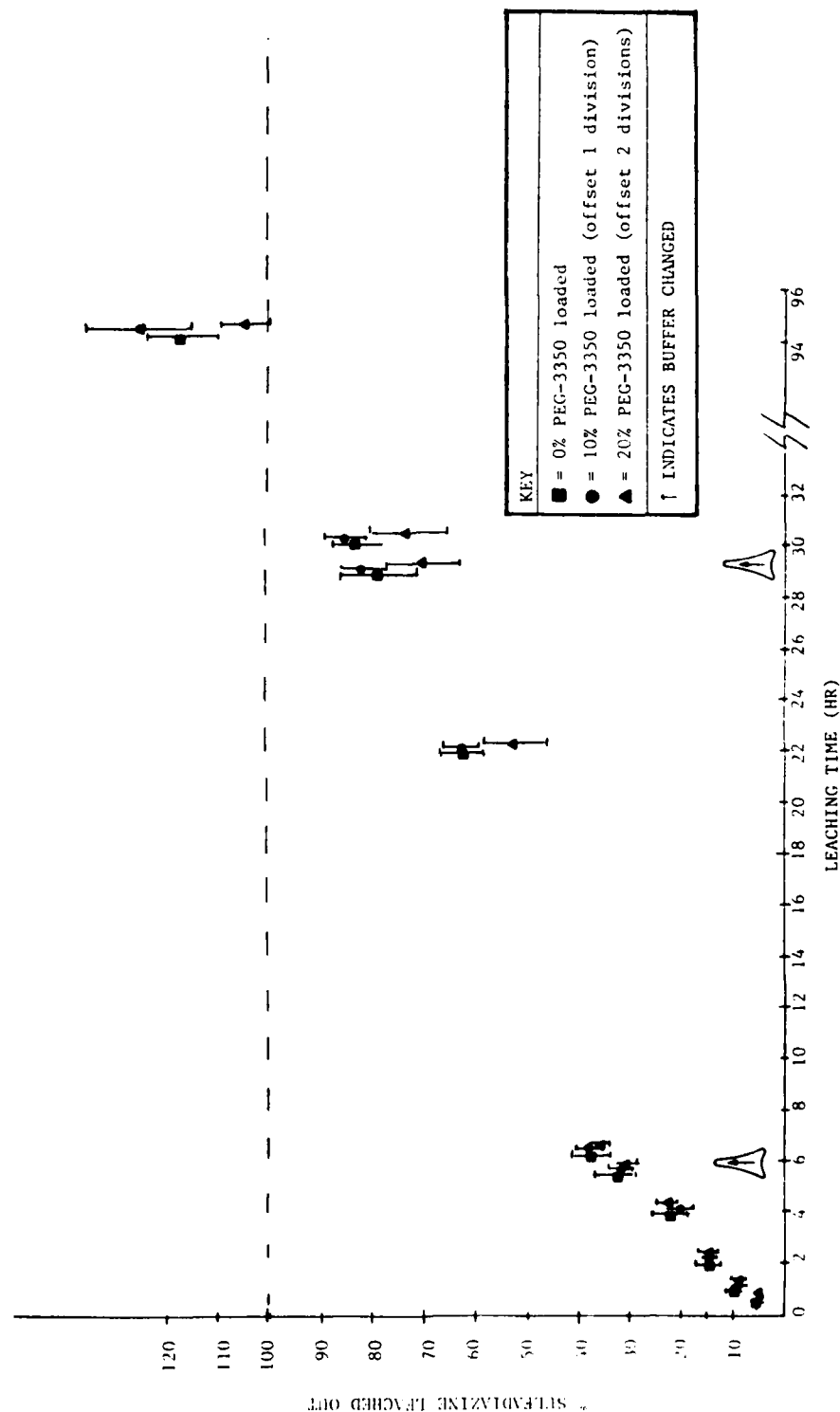
Periodically, as shown in Figure 2.6, samples of leachate were withdrawn and A_{273} vs. H_2O was recorded. (The samples then were replaced to avoid volume changes.) Flasks were swirled irregularly during this time, but were mostly static and unstirred.

As the sulfadiazine dissolved and leached out, the gel films lost their opacity, with the thinnest portion clearing first. By the third day all the films had completely cleared, and the leaching was deemed complete. All films remained totally adherent to the flask bottoms; thus, leaching took place through the upper surface of the films only, modelling release onto a well-wetted body surface.

An assay wavelength of 273 nm was selected as having the best signal:noise ratio between the sulfadiazine UV peak [A_{251} (10^{-2} mg/ml) = 0.973, A_{273} (10^{-2} mg/ml) = 0.362] and the background of the control PVIPAA leachate (at 4 hr leaching time, A_{273} = 0.012, A_{250} = 0.101).

The release rate is approximately linear for the first 40% of release (up to 7 hours); after that it diminishes due to two factors: 1) the films leach all the sulfadiazine from their thinnest parts, hence the area effectively leaching drops considerably; and 2) as the sulfadiazine leaches from the portion of the gel near the surface, and must dissolve and diffuse from deeper portions of the gel, the gradient and hence the transport rate diminish.

Figure 2.6
Leaching of Sulfadiazine from PVIPAA/Glutaraldehyde Films Containing
Varying PEG-3350 Levels into pH 7.0 Buffer
(Graph 017984-A)



We hypothesize that the endpoint leaching values of slightly >100% may arise as follows. The analysis supposes that the sulfadiazine is dispersed and delivered from the vials exactly in proportion to the amount of gel medium; this may not be strictly true. Assuming complete delivery of the sulfadiazine from the vials gives an average delivery of 96 \pm 5% for all nine sulfadiazine films together. PEG-3350 itself exhibits negligible absorbance at 273 nm, and other studies have shown negligible effect on the stability of the gel caused by its presence (at up to 20% of casting-weight), thus the single 20% PEG-3350 control serves for all experimental films. Sulfadiazine is stable at pH 7.0; thus, no aging control on it is necessary.

The therapeutic significance of the sulfadiazine release is as follows: over the 3-day period, a total of 50 mg/40 cm² or 1.25 mg/cm² was released. With higher sulfadiazine loadings than the 1:12 sulfadiazine: PViPAA used in this experiment, the initial release rate of ~6%/hr x 1.25 mg/cm² or 0.07 mg/cm²/hr could be maintained for many days.

2.12.2 Leaching of Penicillin

The effect of the presence of polyethylene glycol, molecular weight 3350 [PEG-3350] on the leaching of sodium benzyl penicillin (penicillin-G) from PViPAA/glutaraldehyde/PEG-3350 films into pH 7.0 buffer, was tested as follows. Fifteen individual films were prepared and cast with formulations as shown in Table 2.14.

Table 2.14

PViPAA/PENICILLIN FILMS

FILM NUMBERS	1 - 3 and 3A	10	4 - 5 and 6A	11	7 - 9 and 9A	12	13
PViPAA	0.60 g	0.60 g	0.60 g	0.60 g	0.60 g	0.60 g	0
PEG-3350	0	0	0.30 g	0.30 g	0.60 g	0.60 g	0
Penicillin-G	200 mg	0	200 mg	200 mg	200 mg	0	200 mg
H ₂ O	2.40 g	2.40 g	2.10 g	2.10 g	1.80 g	1.80 g	~3 ml
2.5% Glut. Solution	0.25 ml	0.25 ml	0.25 ml	0.25 ml	0.25 ml	0.25 ml	0

These formulations were prepared in three dram vials by dissolving the PViPAA and PEG-3350 in the H₂O. The NaPenicillin-G was added and dissolved. When ready to cast, for each vial, the glutaraldehyde was injected, the vial vortexed ~8 sec, and the contents cast into a flask (as described for sulfadiazine, above). The gels all set to no-flow in about two min. (The NaPen G causes a pH increase from 8.1 to 8.25.) Vial #13 was thoroughly rinsed into its flask, serving as a penicillin-degradation control solution. After 2-1/2 hours setting time, leaching was started, as described previously; the incubation temperature was 37°C for this experiment.

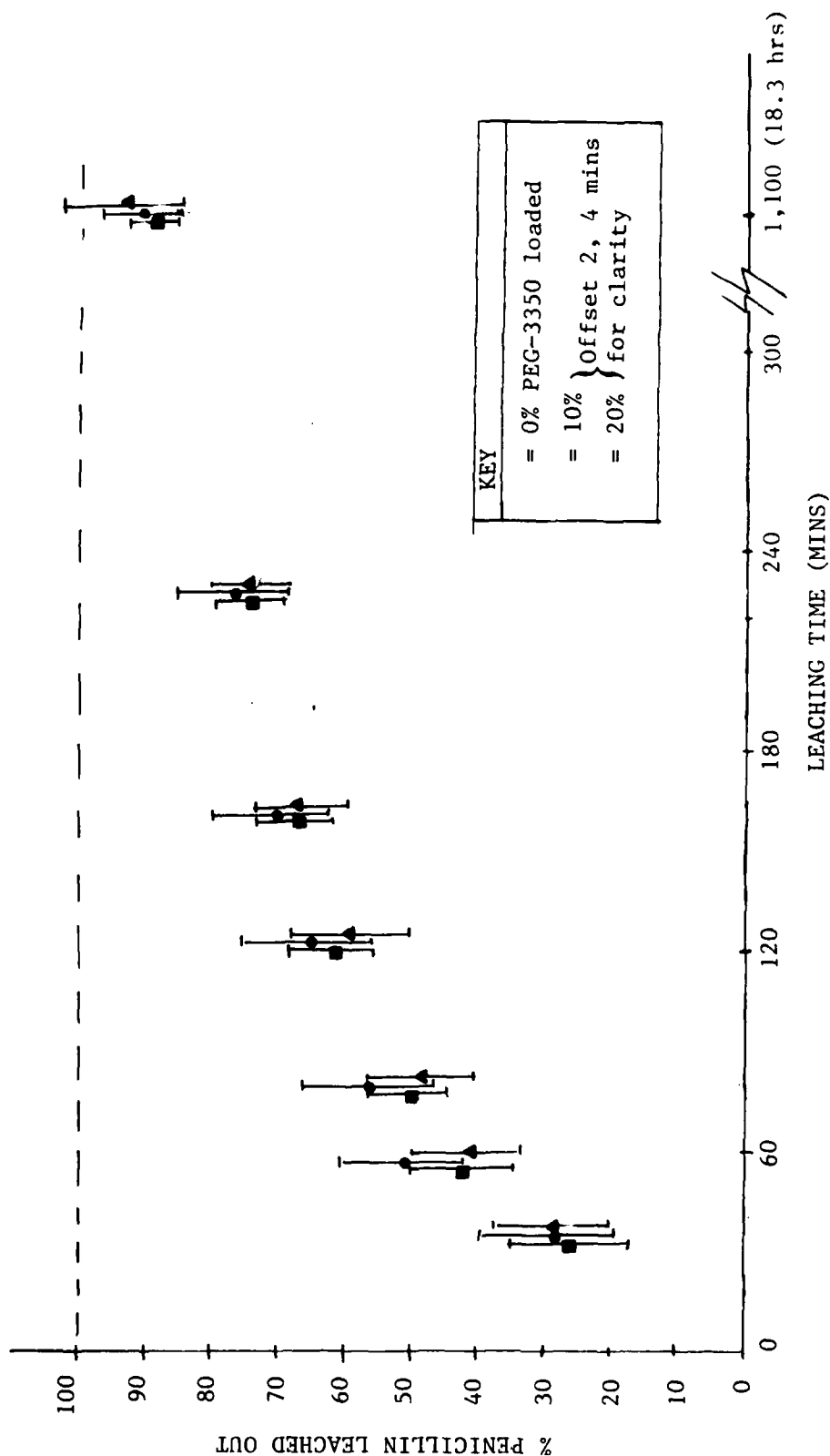
Samples were checked for Absorbance at 259 nm periodically, using the procedure described in 2.12.1. Because this drug is highly water soluble, it dissolved in the film and the castings were clear. Penicillin leached more rapidly from the films than did sulfadiazine; also, for the same reason, no buffer changes were necessary.

An assay wavelength of 259 nm was selected as optimizing the signal background ratio for detecting penicillin in the presence of leached-out PViPAA. The background is far from negligible, comprising 70% of initial and 50% of the final A₂₅₉ readings; thus, the assay requires accuracy of both experimental and control readings, somewhat of a problem when typical standard deviations are 10 - 20% of measurement size. Nevertheless, consistent series of data readings were observed, with the primary cause of standard deviation in the sets of 4 films being different, but smooth, rates of leaching from the individual films. Such differences would arise from variations in film thickness, as noted in 2.12.1.

Experimental data was treated as described previously, and is displayed in Figure 2.7. As can be seen, leaching of the penicillin-G is rapid for the first 50% of release (up to 80 min) and continues at a diminishing rate thereafter.

Figure 2.7

Leaching of Sodium Benzyl Penicillin (Penicillin-G) from PVI-PAA/Glutaraldehyde Films Containing Varying PEG-3350 Levels into pH 7.0 Buffer
(Assayed by A_{259} , Against Control PVI-PAA/PEG Films and NaPen-G Standard)
(Graph 017994-A)



An endpoint leach of ~90% of the loaded drug is observed. This could represent a slight adsorption on the gel, but more likely is an artifact due to slightly high A₂₅₉ of the controls, and thus a slightly low calculated release. The controls may be expected to have slightly high A₂₅₉, because they are cast from a slightly lower pH formulation than the penicillin containing gels, which will affect gel firmness, setting time, and probably swellability.

Total amount of drug released is:

$$\frac{200 \text{ mg NaPenG} \times 90\%}{\sim 40 \text{ cm}^2} = 4.5 \frac{\text{mg}}{\text{cm}^2}$$

This figure has the following therapeutic significance:

$$4.5 \frac{\text{mg}}{\text{cm}^2} \times \frac{1070 \text{ units}}{\text{mg}} = \frac{7,500 \text{ units activity}}{\text{cm}^2}$$

over their lifetime of <1 day. This dosage could most likely be increased several fold, but the release time could not be extended without insolubilizing the drug (e.g., a metal salt).

Section 3

DEVELOPMENT OF A GELATIN-BASED WIPE-ON WOUND COVERING

3.1 Initial Experiments with Gelatin

Experiments were conducted with two grades of gelatin obtained from Geo. A. Hormel & Co. The first is Hormel GP-4, 144 Bloom, Lot No. OKD 333-1. This is an edible collagen hydrolysate capable of forming reversible gels at room temperature. A parallel series of experiments was initiated with another Hormel product, Polypro 5000 (E.D.P. No. 46505, Lot No. 20323). Polypro 5000 is also a water soluble, edible, collagen hydrolysate of apparently lower molecular weight. It forms solutions which do not gel at room temperature. Attempts to crosslink it with aldehydes were unsuccessful. The material may be useful as an additive to the GP-4 to prevent room temperature gelling, but the appropriate ratio of GP-4 to Polypro which will permit crosslinking but which will not gel at room temperature has not yet been determined.

The purpose of these experiments was to develop a method and formula for producing flexible, water insoluble films from water soluble gelatin. Gelatin is a protein polymer which may be crosslinked by a variety of materials, including aldehydes. Two aldehydes were chosen for initial experiments: formaldehyde and glutaraldehyde. The former is a monoaldehyde (HCHO), the latter is a dialdehyde ($\text{OHC}-(\text{CH}_2)_3-\text{CHO}$). Reaction of proteins with aldehyde results in production of crosslinked water insoluble chains.

Initial experiments were conducted with glutaraldehyde as a crosslinking agent. When glutaraldehyde is present in high concentrations, gelatin is insolubilized immediately. The amount of glutaraldehyde needed is sensitive to the experimental conditions. A wipe-on system employing a crosslinking agent must permit time for thorough mixing of the ingredients as well as time for applying the mixture to the wound. For the broad spectrum of initial experimental work, a slow, crosslinking reagent was

desired. All further experiments were performed with aqueous formaldehyde. With this reagent, the crosslinking reaction was sufficiently slow under varying conditions to permit thorough mixing and spreading.

Gelatin solutions may be made by warming a mixture of gelatin and water. Such a stock solution was made containing 16.7% by weight of gelatin (10 g gelatin/50 ml H₂O). The solution remains liqu'd above 35°C, but "sets" reversibly when the temperature falls to 25°C.

A series of gelatin/formaldehyde mixtures were made, some containing plasticizer, in order to determine an optimal formula. The compositions of the stock solutions from which these were made are presented in Table 3.1. Films were prepared by mixing 10 ml of gelatin stock solution with varying quantities of plasticizer (glycerol) and cross-linking agent (37% aqueous formaldehyde). Three stock solutions were used in these experiments with calcium chloride added to prevent gelation. These will be described.

Table 3.1
STOCK SOLUTION COMPOSITIONS

STOCK	COMPOSITION	
	GELATIN, GP-4	DIST. H ₂ O
011287	10.0 g	50.0 ml
011290-A	20.0 g	50.0 ml pH adjusted to 7.2 with 1.0 N NaOH
011294	20.0 g	50.0 ml
011296	20.0 g	50.0 ml + 1.0 ml glycerol

Film compositions and observations on these films are presented in Table 3.2. From this work, a composition suitable for film formation can be recommended. This is as follows:

Table 3.2
FORMULATION OF INSOLUBILIZED GELATIN
FILM: HORMEL GELATIN GP-4 144 BLOOM

SAMPLE NO.	COMPOSITION	OBSERVATIONS ON CROSS-LINKING	FILM CHARACTERISTICS
011287-A	10.0 ml stock 011287 1.0 ml HCHO (1)	Very slow to cross-link; incomplete after 20 min.	Brittle after 24 hrs, difficult to remove from dish.
011287-B	10.0 ml stock 011287 1.0 ml HCHO 1.0 ml Glycerol	As for 011287-A, possibly slower after 0.5 hrs, this was still tackier than 011287-A.	Supple and flexible after 24 hrs.
011288-A	10.0 ml stock 011287 2.5 ml HCHO	Slow gelling, over 10 min.	Less brittle than 011287-A. Can be folded. Feels harder to the touch than 011287-B.
011288-B	10.0 ml stock 011287 2.5 ml HCHO 1.0 ml glycerol	Slightly slower to gel than 01288-A.	Supple and flexible after 24 hrs. May be a bit More elastic than 011287-B.
011290-B	Stock 011290-A 10.0 ml 28.6% (2) 2.5 ml HCHO 1.0 ml glycerol	Much faster to cross-link than 011290-C or -D.	Original Dia. = 3.50" Final Dia. = 2.70, 2.60" After 18 hrs: Diameter shrinkage: 24.3 + 2.0 % Thickness 0.040 + 0.005" (SD) (edge) 0.045 + 0.003" (SD) (center) Film is flexible, dry, slightly elastic.
011290-C	10.0 ml stock 011294 2.5 ml HCHO 1.0 ml glycerol	Slow to cross-link.	After 18 hrs: No Shrinkage of diameter. Thickness 0.027 + 0.006" (SD) (edge) 0.020 + 0.004" (SD) (center) Film is attached to dish around perimeter, but mostly pulled away from bottom. Some cracking. Film is strong, flexible and slightly elastic. Less flexible than B.
011290-D	10.0 ml stock 011294 1.0 ml HCHO 1.0 ml glycerol	Slow to cross-link	After 18 hrs: Completely adhered to dish. Peeled away without tearing. Strong flexible but not elastic. Less flexible than B. Thickness 0.024 + 0.007" (SD) (edge) 0.021 + 0.003" (SD) (center)
011290-E	Stock 011290-A 10.0 ml 28.6% (3) 1.0 ml HCHO 1.0 ml glycerol	About as fast to cross-link as 011290-B.	After 18 hrs: About half adhered to dish. Strong, slightly elastic, flexible, but less flexible than 011290-B.
011294-A	10.0 ml stock 011294 1.0 ml glycerol 1.0 ml HCHO	Cross-linked in ~2 mins.	After 24 hrs: Less flexible than 011294-B, -C, -D, although strongest.
011294-B	10.0 ml Stock 011294 2.0 ml glycerol 1.0 ml HCHO	Cross-linked in ~2 mins.	After 24 hrs: Very flexible with more strength than 011294-C or -D. More flexible than 011294-A
011294-C	10.0 ml Stock 011294 3.0 ml glycerol 1.0 ml HCHO	Rapid cross-linking, but remained tacky to the touch for at least 15 - 20 mins. Tackiness disappeared after warming on hot water bath.	After 24 hrs: Less tear strength than 011294-A, and -B. Good flexibility.
011294-D	10.0 ml Stock 011294 3.0 ml glycerol 2.5 ml HCHO	Cross-linked in ~2 mins.	After 24 hrs: Similar to 011294-C.

Stock solution: 28.6% by weight of GP-4 gelatin in distilled water.

To make film: Stock solution: 10.0 volumes
glycerol: 2.0 volumes
37% aq. HCHO: 1.0 volume

This combination gives a crosslinking time which is rapid yet permits sufficient leeway for mixing application. The film has strength, and flexibility. It is unnecessary to adjust pH to achieve this rapidity of crosslinking. The film remains flexible and strong indefinitely.

The difficulty with this formulation is that the stock solution undergoes reversible gelling at room temperature. Stock solution 011287 was warmed in a water bath. The solution was entirely liquid at 35°C. On cooling, it had gelled completely at 25°C. Several experiments were conducted to evaluate additives as a means for prevention of gelling. Many materials, both organic and inorganic, are known to have a peptizing effect on protein solution⁽¹⁾. These experiments are summarized below.

Effect of pH on Gelatin. pH was varied from its unadjusted value (5.7) to 7.2 by addition of NaOH and to 4.0 by addition of HCl. No effect on gelation was observed, although the gel may have been more easily disrupted at the higher pH.

Addition of Ethanol. Ethanol acts as a precipitant for dissolved gelatin.

Glycerol. Glycerol acts as a plasticizer for the insolubilized film, but does not prevent gelation. Addition of up to 3.0 ml/10 ml of 011294 Stock had no effect.

Calcium Chloride. Calcium chloride dihydrate was added in increasing amounts to Stock solution 011296. The CaCl₂ was dissolved in 1.5 ml H₂O and added to 12.0 ml of stock. After observation on gelation, 1.0 ml of HCHO was added to determine the effect on the crosslinked material.

¹ Encyclopedia of Polymer Science and Technology, Vol. 7, p. 452.

3.2 Equilibrium Properties of Crosslinked Gelatin Films

The purpose of these experiments was two-fold: first, to measure the quantity of water loss from the insolubilized films immediately after formulation; and second, to measure the rate of water vapor transmission through these films after they have reached an equilibrium moisture content.

One formulation, plasticized with glycerol, was tested. Samples were made by incorporating formaldehyde and glycerol into aliquot portions of a stock solution. This stock solution (014958-1) was made by dissolving 20.0 g of Hormel GP-4 gelatin in 50.0 ml of distilled water. Five films were cast in Petri dishes from this stock. Three Petri dishes had diameters of 8.9 cm each, two of 9.7 cm each. The film formulation was as follows:

Stock solution 014958-1	10.0 ml
Formaldehyde, 37% aq.	1.0 ml
Glycerol	1.0 ml

A record was kept of weight loss until equilibrium was reached. This is reported in Table 3.3. At equilibrium, films had lost $62.6 \pm 1.1\%$ of their initial weight.

Film thicknesses were also measured at equilibrium. The mean film thickness of the 8.9 cm diameter films was 0.52 ± 0.08 (SD) mm. The mean film thickness of the 9.7 cm diameter films was 0.33 ± 0.07 (SD) mm. Dimensional data for each film are also recorded in Table 3.3.

The equilibrium water content may be estimated reasonably well. The total volume of gelatin solutions is additive: in work to be reported below, a volume of 170 ml was recorded for a solution of 48 g of GP-4 gelatin in 120 ml of water. Thus, the solids content of 10 ml of Stock 014958-1 will be $(2/7)10 = 2.86$ g. The glycerol content (1.0 ml) is therefore 1.26 g. The total non-volatile weight, excluding crosslinking moieties from the

Table 3.3

WEIGHT LOSS AND DIMENSIONS OF CROSSLINKED GELATIN FILMS

TIME (hrs)	WEIGHT LOSS IN GRAMS OF FILMS				
	014958-1A	014958-1B	014958-1C	014958-1D	014958-1E
1.5	1.57	1.44	1.44	1.58	1.54
65.5	7.11	7.36	6.77	6.10	6.29
71.5	7.05	7.33	6.75	6.07	6.26
Final Film wt.	4.05	4.20	3.86	3.87	3.88
% wt. loss	63.5	63.6	63.0	61.1	61.7

Mean Film wt. = 3.97 ± 0.15 (SD) g

Mean % wt. loss = 62.6 ± 1.1 (SD) %

Film Thickness (mm)	0.52 ± 0.05	0.60 ± 0.03	0.45 ± 0.07	0.40 ± 0.01	0.27 ± 0.01
Film Diameter (cm)	8.9	8.9	8.9	9.7	9.7

Mean Film Thickness (8.9 cm dia) = 0.52 ± 0.08 (SD) mm

Mean Film Thickness (9.7 cm dia) = 0.33 ± 0.08 (SD) mm

formaldehyde is therefore $2.86 + 1.26 = 4.12$ g. The observed mean weight of these films at equilibrium was 3.97 ± 0.15 g. Therefore, we conclude that the equilibrium water content of these films is negligible.

3.3 Water Vapor Transmission Through Equilibrated Crosslinked Gelatin Films

After films 014958-1A through -1E had equilibrated in air, the rate of water vapor transmission through these was measured. The apparatus employed was the Payne Permeability Cup, with 3.5 cm diameter. These cups are equipped with a flange extending around the cup rim and a washer with internal and external diameters matching the cup rim diameter and the flange diameter. The cups were filled with 10.0 ml of distilled water each. Films were sealed to the flange and washer with high vacuum silicone grease to insure no air or moisture leaked past the films. Measurements of weight were taken periodically. Between weighings, cups were stored at 35°C. The weights of water lost through these films are shown in Table 3.4.

From data reported in Table 3.4, it is possible to calculate the rate of water vapor transmission through each film. The rate is highest for the thinnest films. It is also useful to calculate the specific rate of loss (independent of thickness). This calculation is made by use of the following formula:

$$R = \frac{w\ell}{tA},$$

where: R = rate of water vapor transmission $\text{g}\cdot\text{hr}^{-1}\cdot\text{cm}^{-1}$;

w = water loss, g, in time, t ;

t = time, hr;

A = film area, cm^2 ; and

ℓ = film thickness, cm.

Specific rates, calculated for each increment of time for each film, are reported in Table 3.5. It is apparent that this rate is not constant for

Table 3.4

WATER VAPOR TRANSMISSION THROUGH CROSSLINKED GELATIN FILMS AT 35°C
(Film Area = 9.621 cm²)

TIME (hrs)	WEIGHT LOSS, GRAMS THROUGH FILMS				
	014958-1A	014958-1B	014958-1C	014958-1D	014958-1E
17.5*	2.1070	1.7186	2.6511	-----	-----
18.8	-----	-----	-----	2.709	2.157
25.0	3.1947	2.59 9	3.6529	-----	-----
41.0**	4.8955	4.3652	5.7890	-----	-----
43.5	-----	-----	-----	5.420	4.168
47.8†	5.5135	5.2472	6.7460	-----	-----
Mean Thickness (mm)††	0.574 + 0.039	0.762 + 0.031	0.508 + 0.051	0.442 + 0.034	0.335 + 0.070

* All films are buckled inward. Films -1A and -1B are more uneven than -1C, which is more smoothly concave inward.

** All films are smoothly concave inward.

† Film -1A is almost flat. Films -1B and -1C are concave inward.

†† Film thickness after measurements. Note that these values differ from equilibrium values.

Table 3.5
RATE OF WATER VAPOR TRANSMISSION*
(g·hr⁻¹·cm⁻¹)

TIME	014958-1A	014958-1B	014958-1C	014958-1D	014958-1E
17.5*	7.183 x 10 ⁻⁴	7.778 x 10 ⁻⁴	7.999 x 10 ⁻⁴	-----	-----
18.8	-----	-----	-----	6.620 x 10 ⁻⁴	3.995 x 10 ⁻⁴
25.0	8.652	9.264	7.053	-----	-----
41.0	6.342	8.758	7.049	-----	-----
43.5	-----	-----	-----	5.042	2.835
47.8	5.422	10.273	7.431	-----	-----

Mean Value: 6.981 ± 1.945 (SD)

Note: These values are calculated for each increment of weight loss and time. Ex.: the rate of water vapor transmission reported for film -1A at 41.0 hrs was calculated as follows:

$$R = \frac{wl}{\tau a} = \frac{(4.8955 - 3.1947)(0.0574)}{(41.0 - 25.0)(9.621)} \quad 6.342 \times 10^{-4}$$

* Using film thickness after measurements.

all film thicknesses. The specific rate of transmission increases with increasing thickness.

3.4 Sustained Release of Phenolphthalein, a Soluble Model Compound, from Crosslinked Gelatin Films

Protective barriers over burned skin should minimize water loss, but not prevent it entirely. Results reported herein suggest that crosslinked gelatin may be a simple and effective means of approximating the normal rate of water loss through skin.

A second function, however, is suggested by the susceptibility of wounds, including burns, to sepsis. Microorganisms trapped between the burned surface and the barrier can proliferate in such an environment. It is therefore desirable to incorporate an antibiotic into the wound covering.

We tested the feasibility of the concept by including in the formulation phenolphthalein, a soluble and easily identifiable indicator of molecular weight 318.33. A film was prepared and allowed to equilibrate as previously described. It was then immersed in distilled water and held in a shaker bath at 37°C. Aliquot samples of the water were removed periodically and the phenolphthalein contents analyzed spectrophotometrically as a function of time.

The film (Film 014959) was cast in a 9.7 cm diameter Petri dish. The recipe for that film was as follows:

Stock Solution 014958-1	8.0 ml
Glycerol	1.0 ml
Phenolphthalein Solution	1.0 ml
37% aq. Formaldehyde	1.0 ml

To make the phenolphthalein solution, 0.10 g of the indicator was dissolved in 10.0 ml of ethanol and then diluted to 30 ml with distilled water. Thus, the indicator concentration was 3.33 g/l. A spectrum was

taken of the phenolphthalein between 450 and 580 nm. To do this the phenolphthalein solution was diluted with 0.1 N NaOH in two steps to a final concentration of 3.41×10^{-2} g/l (0.5 ml to 3.05 ml and 0.2 ml of this to 3.2 ml). The spectrum revealed a single maximum at 546 nm with a specific absorbance of 3.66×10^4 ml·g⁻¹·cm⁻¹.

After casting, the film was allowed to air dry prior to taking any further measurements. The film was immersed in 200 ml of distilled water and the flask was placed in a gently agitated shaker bath at 37°C. Periodically 0.5 ml aliquot samples were withdrawn and diluted with 4.0 ml of 0.1 N NaOH. The absorbance, A, at the wavelength maximum was measured and the quantity of phenolphthalein in solution calculated from the following equation.

$$w = AV/\epsilon d$$

where: w = weight of phenolphthalein, mg;

A = absorbance;

V = volume of solution, 200 ml;

ϵ = specific absorbance, 3.68×10^4 ml·g⁻¹·cm⁻¹; and

d = path length = 1.0 cm.

These data are presented in Table 3.6.

Although recovery of phenolphthalein is only about 15% in two days with half of that released within the first three hours, this experiment clearly demonstrates that sustained release from gelatin is possible. The remaining phenolphthalein is retained. Quite possibly, some of it has been covalently attached to the crosslinked polymer. It should be stressed, however, that this experiment was conducted with a dry film; in actual use, the water content of the film will initially be much higher. This will probably facilitate drug release in the absence of chemical alteration of the drug.

Table 3.6

SUSTAINED RELEASE OF PHENOLPHTHALEIN

TIME (hrs)	ABSORBANCE AT 546 mm	mg. RELEASED	% RELEASED
2.75	0.049	0.27	8.1
21.25	0.096	0.52	15.6
46.75	0.092	0.50	15.0

3.5 In Vivo Adherence Evaluation of the Gelatin Based Wipe-on Burn Covering

Two formulations were evaluated on burned rats using unburned rats as controls. Wistar (CD-1) rats of either sex weighing between 150 and 200 grams were obtained from Charles River Breeding Laboratories.

Stock solutions of each formulation were made up according to the following recipes.

<u>Stock Solution No.</u>	<u>014969-1</u>	<u>014969-2</u>
Hormel GP-4 Gelatin	48.0 g	48.0 g
Distilled Water	120.0 ml	120.0 ml
Glycerol	17.0 ml	34.0 ml

The volume of gelatin solution before addition of glycerol was in each case ~170 ml, allowing an estimate of the gelatin content of films to be made.

Twenty four rats were used in this experiment. Twelve were reserved for controls, twelve were burned. The latter are termed the experimentals. Six controls received formulation 014969-1, six received formulation 014969-2. A similar division was made of the experimentals. In order to observe difference in ease of removing the burn coverings, six controls and six experimentals were stripped of their coverings on the same day of application about two hours later. Coverings were removed from the remaining animals about 24 hours after application.

Animals were prepared for the experiment as follows. All were anesthetized with 50 mg/kg of Nembutal™. Their backs were shaved and then depilated with Nair™. Burns were administered by pipeting 1.5 ml of ethanol into an elliptical dam (major axes: 3 cm x 7 cm) held against the rat's back. The alcohol was ignited and allowed to burn until none remained. This technique achieves a reasonably uniform second to third degree burn. After burning, 10.0 ml of the stock solution was rapidly mixed with 1.0 ml of 37% aqueous formaldehyde. The still liquid mixture was brushed evenly over the burned area and a strip of gauze lightly pressed into it. To insure that the gauze was thoroughly imbedded in the gelatin, some of the gelatin solution was brushed over the gauze. The gauze was Hospital Brand™, 44 x 36 mesh USP Type 1. Control rats were covered in an identical manner.

Coverings were removed from 12 animals after 2 hours. These 12 included 6 controls, 3 of which had received Formulation 014969-1 and 3 of which, Formulation 014969-2. Of the remaining 6 experimentals, 3 had received Formulation 014969-1 and 3, Formulation 014969-2.

Twenty-four hours later, the remaining twelve animals were stripped of their coverings. Treatment regimes were as above. Prior to cover removal, animals were housed in individual cages and allowed food and water ad libitum. Overnight, only one animal, C-9, had pulled its cover off and was lost to the experiment.

Coverings were removed by the method described and illustrated in Dynatech Report No. 2165 (Dynatech's Final Report on Contract N00014-81-C-0468) dated May 7, 1982. Anesthetized animals were held on a specially constructed board mounted in an Instron Tensile Tester as shown in Figures 9.1 - 9.3 of that report. Table 3.7 presents the mean adherences of the coverings on the test animals.

Gelatin films adhered well to the contours of the animals backs, and were removable with no injury to either the burned or unburned tissue. The greatest mean adherences were 0.240 lb/in., found with films removed from burns after 24 hrs, and 0.271 lb/in. with one set of films

Table 3.7

MEAN ADHERENCE OF GELATIN FILMS, LBS/IN*

FORMULATION	CONTROL		EXPERIMENTAL	
	014969-1	014969-2	014969-1	014969-2
2 hrs	.049 \pm 0.33	0.059 \pm 0.037	0.165 \pm 0.046	0.122 \pm 0.53
24 hrs	.0175 \pm 0.038	0.271 \pm 0.070	0.240 \pm 0.109	0.162 \pm 0.079

Note: In all cases, these rats were used in each determination, except for the 24 hr pull of control formulation 014969-1, for which two animals were used.

* These values are corrected from Table 3.5 in Dynatech Report #2229.

removed from unburned skin. Differences between the 24-hour controls and experimentals do not appear to be significant. At 2 hours, however, the experimentals of both formulations showed greater adherence than did the controls of either formulation. Although controls at 24 hours of either for mulation adhered more strongly than did the controls at 2 hours, this dif- ference was much less pronounced with the experimentals.

3.6 Prevention of Reversible Gelation of Gelatin

Hormel GP-8 Gelatin, 252 Bloom, Lot No. OKD 325-1 was used in this experiment to prepare solution 014991. This solution contained 7.25 of the GP-8 dissolved in 50 ml of warm distilled water. After the gelatin had all dissolved, the solution was made acid with 0.25 ml of concentrated hydrochloric acid. The solution was brought to reflux and periodically the gel temperature was measured by removing an aliquot portion, and cooling slowly. The temperature at which a coherent gel formed on cooling was taken as the gel temperature. Table 3.8 is a record of observations made on 10 ml aliquot samples.

A 10 ml aliquot of the boiled GP-4 solution was removed after 185 minutes of reflux. Three successive 2 ml portions of 37% aqueous

Table 3.8

GELATION OF ACIDIFIED GP-4 SOLUTION AFTER REFLUX AT 100°C

TIME	GEL TEMP °C	COMMENTS
0	> RT	
15	~ 22	
35	19 - 20	
50	20 - 21	
115	~ 17	Gels flows slightly
185	~ 13	At 14°C, a viscous liquid

formaldehyde were added, but the solution did not crosslink until it had been heated. Even after heating, it was not as rigid as would be expected, but resembled instead, a very viscous gel.

To test the assumption that the lack of rigidity was due to excessive dilution accompanying addition of the 6 ml of HCHO, a second portion of solution 014991 was removed. The volume of this aliquot sample was ~ 15 ml, at pH 2.5. The pH was adjusted to 6.8 with aqueous NaOH before proceeding. Two successive 2.0 ml portions of HCHO were added, but no crosslinking was observed. On heating, however, it did crosslink, forming a more satisfactory rigid gel than did the former sample.

To this crosslinked gel 20 ml of water were added; after boiling for 10 minutes, the gel had not dissolved proving that crosslinking had indeed occurred.

The results of this experiment indicate that gentle hydrolysis of gelatin can reduce the gel temperature sufficiently below room temperature without destroying its capacity to crosslink to a rigid structure. Control of hydrolytic conditions is important: time, temperature, solids content, and acidity are parameters which must be controlled.

Other experiments conducted with GP-8 solutions of the same concentration bear this out. In one case, 20 ml of concentrated HCl were added to 100 ml of gelatin solution. After refluxing at 100°C for 15 mins, an aliquot sample did not gel even at 2°C; nor did it crosslink with HCHO. However, prior to adding HCl, the solution had been boiled for ~1.5 hours with no reduction of gel temperature.

It is interesting to note that another Hormel gelatin, Polypro 3000, does not form gels nor does it crosslink. A similar material, adhesive grade collagen hydrolysate derived from fish byproducts and marketed as LePage's original glue, does not gel at room temperature although it easily crosslinks with formaldehyde. The solids content of this material is 50%.

3.7 Sustained Release of Sodium Penicillin-G and Sulfadiazine from Crosslinked Gel Matrices

Films incorporating radiolabeled antibacterials, either penicillin G or sulfadiazine were applied to rats. The purpose of these tests was to demonstrate sustained release of these drugs from the films in vivo. These results are reported in Section 4.

Prior to initiating these in vivo tests, films incorporating the two drugs were prepared and tested in vitro. Analysis of release into the in vitro bath by high performance liquid chromatography (HPLC) made use of the radiolabel superfluous, thus these tests were conducted with unlabeled drugs.

Two stock gelatin solutions, differing in glycerol content, were prepared. These are described in Table 3.9. Each solution was used to prepare two films, one containing penicillin, the other, sulfadiazine. The preparation of each film is given in Table 3.9. The volume of gelatin stocks were chosen to contain equal quantities of gelatin: stock solution 014969-2 contains twice as much glycerol as does stock solution 014969-1. The ratio of weights of the two drugs is the ratio of their molecular

Table 3.9

PREPARATION OF CROSSLINKED GELATIN FILMS CONTAINING ANTI-BACTERIALS

Material	FILM			
	01500-1P	01500-1S	01500-2P	01500-2S
Stock 014969-1, ml	10	10	---	---
Stock 014969-2, ml	---	---	11	11
NaPenicillin G ⁽¹⁾ , gms	0.9997	---	1.0002	---
Sulfadiazine ⁽²⁾ , gms	---	0.7022	---	0.7016
37% aq. HCHO ⁽³⁾ , ml	1.0	1.0	1.0	1.0

COMPOSITES	014969-1	014969-2
Gelatin, GP-4, g	48	48
Distilled water, ml	120	120
Glycerol, ml	17	34

- (1) Sigma Chemical Co. Lot 102F - 0242
- (2) Supplied by Walter Reed Army Institute of Research
(WR-7557 AP, BB 59190, 15817)
- (3) J.T. Baker Chem. Co. Cat. No. 1-2106, Lot 806818

weights. Thus each film contains between 2.57 and 2.59 g of gelatin and 0.00274 moles of drug; that is, ~ 0.001 moles drug/g gelatin.

All films were cast in Petri dishes of 9.7 cm diameter. The drugs were weighed directly into the Petri dishes. About 1 - 2 ml of distilled water was added to each dish prior to adding the gelatin. The penicillin dissolved easily. A paste was formed of the sulfadiazine. Films were allowed to air dry to constant weight in the dishes prior to initiating the in vitro tests. Each dish was warmed for about one minute above the gel temperature to insure that crosslinking had occurred in the presence of the drugs. In no case was reversible gelling observed; the gelatin was satisfactorily crosslinked.

Equilibrium weights were reached in approximately two days. The appearance of the films reflects the drug solubility: Films 015000-1P and -2P, containing the penicillin are clear; films -1S and -2S, into which the sulfadiazine is incorporated, are opaque white.

From each circular disk were cut three 2 cm x 5 cm strips for in vitro testing of release rates. Mean thickness and weight of each strip were recorded. The ratio of strip weight to total film weight allowed calculation of drug contained in each strip.

Each strip was immersed in 25 ml of pH 7 0.05 M $\text{KH}_2\text{PO}_4/\text{NaOH}$ phosphate buffer (Fisher) contained within a 23 mm diameter test tube. Test tubes were held in a 37°C thermostatted shaker bath. Aliquot samples, 2.0 ml each, were removed periodically for analysis by HPLC.

Penicillin solutions (0.5 mg/ml and 0.05 mg/ml) in pH 7 buffer held at 37°C were analyzed in days 0, 1, and 3 following preparation. Fifty μl samples were analyzed by isocratic HPLC with 1.6 ml/min 1:1:: CH_3CN :0.04 M aq. NH_4OAc , on a 30 x 0.46 cm C_{18} 10 μ -packed column. A major peak at a retention volume of 2.66 - 2.75 ml was accompanied by a penicillin degradation product peak at ~ 2.40 ml. This peak became larger with passage of time although estimation of its magnitude was difficult because of incomplete

resolution from the buffer peak. Major peak decay of standards of 0.5 mg/ml and 0.05 mg/ml revealed approximate degradation rates of 6.1%/day and 12.6%/day. The latter value was chosen to correct the in vitro leaching results of penicillin.

Results indicate rapid release of penicillin. After the removal of the first aliquot sample at 4.25 hours, the concentration was already at a maximum. Similar results were found with sulfadiazine release.

Section 4

ANIMAL TESTS

4.1 Introduction

Tests of wipe-on films were conducted using rats as the model animal. The purpose of these tests was to determine if drugs incorporated into the wipe-on formulation would be absorbed through the animals' skin. The radio labeled drugs chosen for these experiments were penicillin G⁽¹⁾ and Sulfadiazine⁽²⁾. Prior to conducting these experiments, the gelatin and PVIPAA films into which these drugs (unlabeled) had been incorporated were applied to two rats to determine if the drugs interfered with adherence. Results of these preliminary adherence tests are reported in Section 4.2 and of the drug absorbance tests in Section 4.3.

4.2 Adherence of Gelatin and PVIPAA Films Containing Penicillin and Sulfadiazine

These initial tests were conducted to verify that adherence to wipe-on films after crosslinking would not be compromised by incorporating either sodium penicillin-G or sulfadiazine into the formulation.

Two gelatin films were prepared as indicated in Table 4.1.

The drug was mixed directly into the gelatin solution. The sodium penicillin-G dissolved easily as expected, and the sulfadiazine easily formed a homogeneous dispersion in the gelatin dispersion. The formaldehyde was added directly prior to application.

The PVIPAA films had compositions reported in Table 4.2. As with the gelatin films, the glutaraldehyde was added just before application.

⁽¹⁾Potassium G-Phenyl [1-¹⁴C] acetamidopenicillanate from Amersham.

⁽²⁾2-[³⁵S] sulphanilamide pyrimidine from Amersham.

Table 4.1

PREPARATION OF GELATIN FILMS FOR PRELIMINARY ADHESION TESTS

Composition	014912-1	014912-2
Gelatin Stock 014969-2(1)	11.0 ml	11.0 ml
Na Penicillin G	0.50 g	---
Sulfadiazine	---	0.35 g
37% aq. HCHO	1.5 ml	1.5 ml

- (1) Gelatin stock 014969-2 consisted of 50 g GP-4 gelatin, 120 ml distilled water and 34.0 ml of glycerol.

Table 4.2

PREPARATION OF PVIPAA FILMS FOR PRELIMINARY ADHERENCE TESTS

Composition	Formulation, Grams	
	016750-A	016750-B
PVP/AA(1)	5.85	5.85
Glycerol	2.30	2.30
Methanol	7.25	7.25
Sodium Dodecyl Sulfate	0.0187	0.0187
HCl (to adjust pH)	(Negligible)	
Glutaraldehyde(2)	0.099	0.099
Distilled Water	4.95	4.95
Na Penicillin G or Sulfadiazine	1.47 --	-- 1.06

- (1) PVIPAA was polymerized anaerobically at 80°C from 347 ml VP, 520 ml EtOH, 10.4 ml allylamine and 2.6 ml t-butyl perbenzoate as initiator.
 (2) Added as 3.5% aqueous solution.

Two rats were used in this experiment. Rat No. 1 received both gelatin films, Rat No. 2 both PViPAA films. The backs of both rats were shaved and depilated with Nair[™], and then washed and dried. The formulations containing penicillin were painted on the right sides of the rats' backs, the ones containing sulfadiazine on the left. A gauze bandage, 1 inch wide, was pressed into the wipe-on before crosslinking and then more wipe-on was applied to the gauze to make sure that it was fully imbedded. Rat No. 1 died within 24 hours of application, so adherence was tested at 28 hour post-application. Rat No. 2 was anesthetized with ether prior to measuring adherence at 52 hours post-application.

Adherence was measured as previously reported (Dynatech Reports No. 2165 and 2229) on an Instron Tensile Tester. Strip chart recordings are included in Appendix A. In all cases the gauze impregnated films were removed intact leaving no film on the rats' backs.

Adherence was calculated from the equation:

$$\text{Adherence (lbs/in)} = AS/LW$$

where: A = Area under curve (in²)
S = Scale (lbs/in)
L = Length of Area (in)
W = Wound Covering Width (in)

Adherence values are reported in Table 4.3.

Adherence of both gelatin based films and PViPAA films are similar. The somewhat greater adherence of PViPAA films may be due to differences in observations on living, rather than dead skin (Rat No. 1 was dead at the time of measurement).

More important is that values for adherence of these films which include the antibiotics are quite similar to those of the gelatin films

Table 4.3

IN VIVO ADHERENCE OF GELATIN AND PViPAA FILMS (lbs/in)

Formulation	Rat No. 1	Rat No. 2
014912-1(3)	0.120	---
014912-2(4)	0.185	---
016750-A(5)	---	0.183
016750-B(6)	---	0.282

(3)Gelatin + Penicillin

(4)Gelatin + Sulfadiazine

(5)PViPAA + Penicillin

(6)PViPAA + Sulfadiazine

without the antibiotics. These observations formed the basis for measurements of the absorbance of radiolabeled drugs from the films through skin.

4.3 Transdermal Absorption Of Radiolabelled Drugs from Gelatin-Based and PViPAA Films

4.3.1 Dilutions of Labelled Drugs

Two drugs were used in this study: [³⁵S]- Sulfadiazine and Sodium-[¹⁴C]-Penicillin G. In each case, the labeled drug was codissolved with unlabelled drugs to achieve the appropriate specific activity. Qualities of materials and radioactivity used in these dilutions are recorded in Table 4.4.

Labelled and unlabelled penicillin were dissolved in ethanol to make a homogeneous solution. Drug was recovered by solvent evaporation. Note that the potassium salt of the labeled penicillin G was used in conjunction with the sodium salt of the unlabeled drug. The high solubility of both, the facile cation exchange, and the large excess of sodium ion minimize the possibility of differential absorption. The sulfadiazines, both

Table 4.4

DILUTION OF RADIOLABELLED DRUGS⁽¹⁾

	Sulfadiazine	Penicillin G	Hydrocortisone
μCi	510	250	250
Label	^{35}S	^{14}C	^3H
Weight Cold Drug, g	9.9890	5.0091	3.0041
Solvent	Water	Methanol	Ethanol
Final Specific Activity $\mu\text{Ci/g}$ (Calculated)	51.06	49.91	83.22
(Measured)	39.01	48.58	79.55

- (1) [^{35}S] Sulfadiazine; Amersham, Cat. SJ 12, Batch 1/82
 Benzyl- ^{14}C -Penicillin, Potassium; Amersham, CFA 244, Batch 74
 ^3H -Hydrocortisone; New England Nuclear, NET-396

Sulfadiazine; Walter Reed Army Institute of Research Batch
 BB 5919015B 17

Sodium Penicillin G; Sigma Chemical, PEN-NA, Lot 102 F-0242

Hydrocortisone; Sigma Chemical, H-4001, Lot 32F-0138.

labeled and unlabeled, were dissolved in water made basic with ammonium hydroxide. The drug was recovered by precipitation with hydrochloric acid.

4.3.2 Preparation of Gelatin Solutions with Drugs

Two gelatin stock solutions were prepared accordingly to previously described methods. To each was added either sulfadiazine or penicillin. The final volume of each was approximately 46 ml. Solution formulae and calculated specific activities are given in Table 4.5.

Table 4.5

GELATIN BASED WIPE-ON RECIPES

Solution	014915	014915-2
Wt. gelatin ⁽¹⁾ , g	12.50	12.50
Vol. dist H ₂ O, ml	30.0	30.0
Vol. glycerol, ml	8.5	8.5
Wt. Penicillin, g	1.826	---
Wt. Sulfadiazine, g	---	1.431
Sp. Act. $\mu\text{Ci/ml}$	1.981	1.581

¹ Hormel GP-4

Conversion of $\mu\text{Ci/gm}$ to DPM for these solutions is as follows:

Calculation of Radioactivity Applied via Gelatin Film

Sulfadiazine/gelatin stock solution:

Weight Sulfadiazine: 1.8263 g
 Vol. gelatin solution: 46 ml
 Specific activity (experimental): 39.01 $\mu\text{Ci/g}$

Applied to rat's back: 5 ml Stock Solution + 1 ml HCHO
 DPM applied = $[1.8263 (5/46)][39.01][2.22 \times 10^6] = 17.19 \times 10^6$ DPM

Penicillin/gelatin stock solution

Weight penicillin: 1.4310 g
Vol. gelatin solution: 40 ml
Specific activity (experimental): 48.58 $\mu\text{Ci/g}$

Applied to rat's back: 5 ml stock solution + 1 ml HCHO
DPM Applied = $[1.4310 (5/46)][48.58][2.22 \times 10^6] = 16.77 \times 10^6$ DPM

4.3.3 Preparation of PViPAA Solutions with Drugs

PViPAA stock solution B016958-1 was made by combining the ingredients shown in Table 4.6. A measured total of 94 g stock solution resulted, which was portioned into 15 vials of 4.4 g net each, with 19.8 g remaining. Dry labelled drugs were added to the vials as in Table 4.7, and mixed.

Table 4.6

PViPAA STOCK SOLUTION FOR USE IN IN VIVO TESTS WITH ANTIBIOTICS

INGREDIENT	WEIGHT(g)	VOLUME (ml)
PViPAA B016442-1	30.7	
Methanol	(38.4)	51
50% wt/vol aq. glycerol	(23.6)	21.5
30% wt/vol SDS		0.315
1N HCl, to pH 6.0		1.2

4.3.4 Preparation of Rats

All rats were anesthetized with Penthrane[™] (Abbott), shaved and depilated with Nair[™] on the day prior to application of the films.

Twelve rats were given alcohol burns prior to application of the covering. An oval metal dam (4.7 x 8.0 cm) was held against the rats'

Table 4.7

AMOUNTS OF ANTIBIOTICS ADDED TO PVIPAA SOLUTIONS AND APPLICATION EFFICIENCIES

DRUG	VIALS NUMBER	SPECIFIC DRY DRUG ACTIVITY $\mu\text{Ci/g}$ (ave.)	DRY DRUG USED (g)	APPLICATION EFFICIENCY (% \pm σ)	ACTIVITY APPLICATION PER RAT (μCi)
Penicillin G	1 - 6	48.58	0.290	89.8 \pm 2.8	12.7 \pm 0.4
Sulfadiazine	7 - 12	39.01	0.260	91.1 \pm 2.8	9.2 \pm 0.3

shaved backs. Into this 1.5 ml of absolute ethanol was introduced by pipet and ignited.

4.3.5 Application of Gelatin-Based Films

The gelatin-based solutions were applied as follows. A 5 ml aliquot sample of either solution 014915-1 or -2 was removed to a 25 ml beaker. To this was added 1.0 ml of 37% aqueous formaldehyde. After thorough mixing, the entire portion was brushed onto the back of a Penthrane anesthetized rat. The amount of radioactivity in each penicillin film was 7.5 μCi , and the amount of radioactivity in each sulfadiazine film was 7.7 μCi . Each rat was kept anesthetized until its film had crosslinked. At that time the film was dusted with talcum powder to minimize damage to the film.

4.3.6 Application of PVIPAA-based Films

Immediately before applying coatings to the rats, 0.7 ml 3.5% EM grade aqueous glutaraldehyde was added to each vial, thoroughly mixed in, and the contents poured onto the rat's back, using a small camel's hair brush to spread the film and help empty the vial. The spent vials were recapped, and they and the applicator brushes (one per drug type) weighed to determine

completeness of application of the gels. The rats therefore received labelled drug activity as indicated in Table 4.7. When the films had gelled to tackiness, they were dusted with talcum (Johnson's Baby Powder) to prevent adhesion to metabolism cage walls and roof (and table top as rats came out of anaesthesia). This addition may have degraded pliability and therefore adhesion by absorbing plasticizer; this point needs further testing.

4.3.7 Maintenance of Rats

All rats were individually housed in metabolism cages, and given food and water ad libitum. Urine and feces were collected daily for each rat over a three-day period after application of the films. Only urine was collected for rats receiving the [^{35}S] - sulfadiazine.

Each rat was numbered and given a three letter designation to indicate the type of antibiotic and the type of film it received and also whether it was burned or unburned. The experimental design is presented in Table 4.8.

4.3.8 Adherence of the Films During the Tests

During the three days of the tests, it was important to monitor the integrity of the films on the rats' backs. Any loss or loosening of the films would reduce the amount of labeled compound available for transfer to the animal. Daily records were maintained on the percent of film remaining on each rat.

Graphical presentation of daily results from burned animals are shown in Figure 4.1 and unburned animal results are in Figure 4.2. The six rats from each group, which are treated with gel films are shown on the left side of each figure; and the six PViPAA-coated rats results are shown on the right side in each figure. Each set of three rats with identical film, antibiotic, and skin treatment is graphed simultaneously. The data-points for each individual rat are connected by a solid line.

Table 4.8

EXPERIMENTAL DESIGN FOR TRANSDERMAL DRUG ABSORPTION

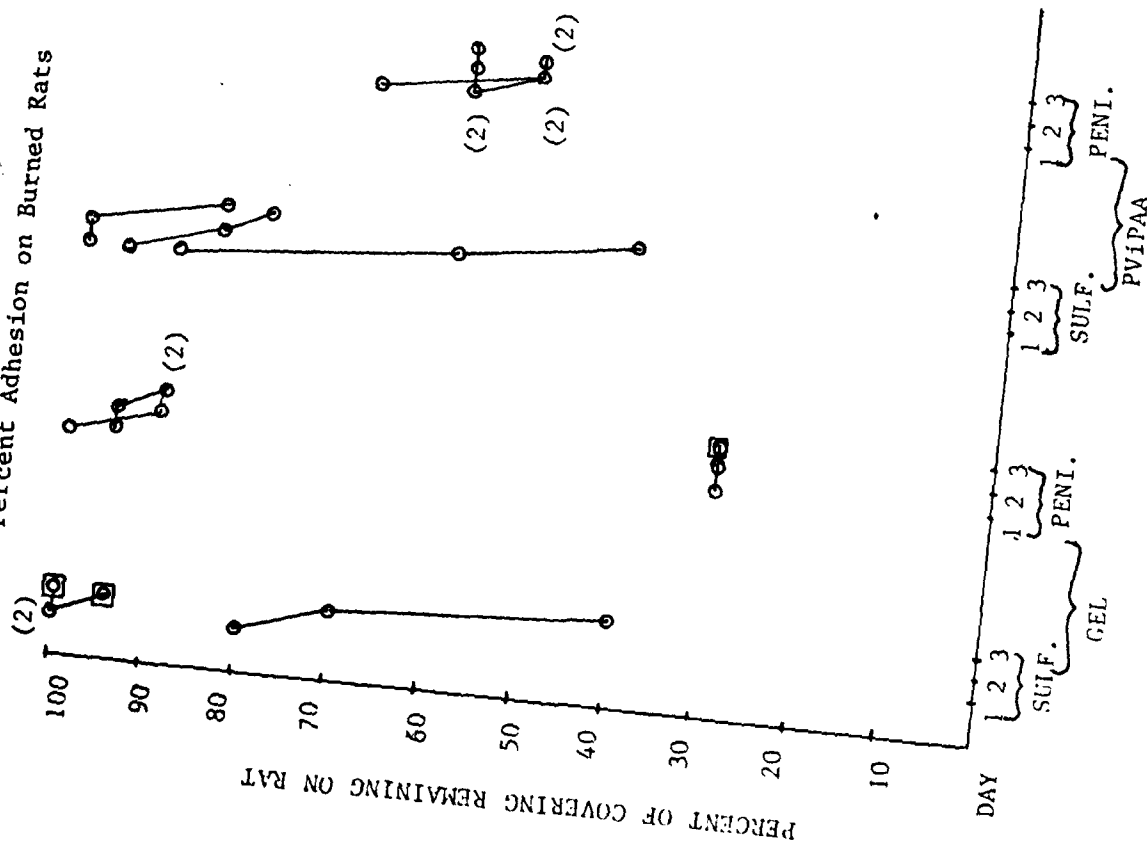
Rat No.	Code ⁽¹⁾	Rat No.	Code
1	SGB-1	13	SPB-1
2	SGB-2	14	SPB-2
3	SGB-3	15	SPB-3
4	SGU-1	16	SPU-1
5	SGU-2	17	SPU-3
6	SGU-3	18	SPU-3
7	PGB-1	19	PPB-1
8	PGB-2	20	PPB-2
9	PGB-3	21	PPB-3
10	PGU-1	22	PPU-1
11	PGU-2	23	PPU-2
12	PGU-3	24	PPU-3

(1) First Letter (drug) S = Sulfadiazine
P = Penicillin

Second Letter (polymer) G = Gelatin
P = PVP/AA

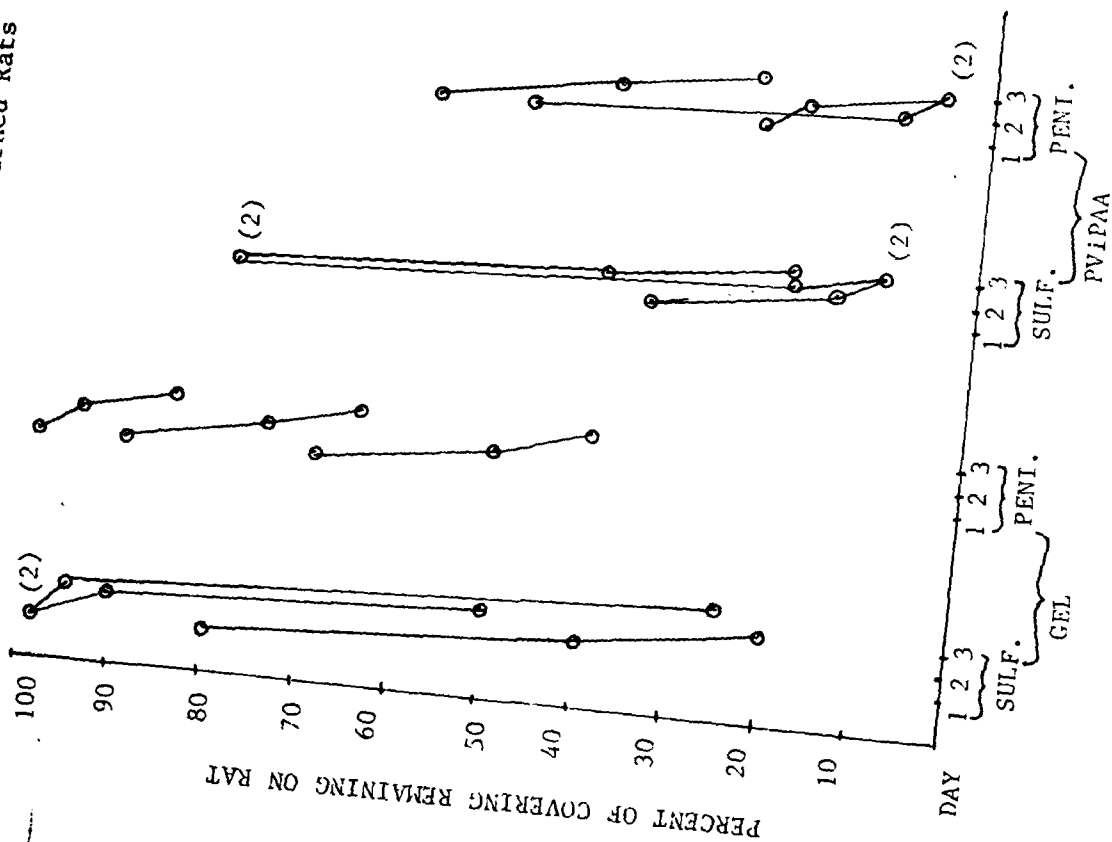
Third Letter (treatment) B = Burned
U = Unburned

Figure 4.1
Percent Adhesion on Burned Rats



○ Daily measurement of percent of film remaining on rat
◻ Represents rat death

Figure 4.2
Percent Adhesion on Un-Burned Rats



Many of the films were loosened or torn by abrasion. The higher adherence of the burned group in comparison with the unburnt may be in part an experimental artifact rather than a film characteristic. The animals were permitted freedom of motion within their metabolic cages and they were able to rub their backs against the cage roof and thus to dislodge the films. The burned animals moved less than did the unburned. There was a much higher loss of film from unburned rats. Hair regrowth was observed in some of these animals and this also may have been a contribution to lack of adhesion.

Of the 12 burned rats, 3 experienced severe film loss. One film became dislodged so that only 30% remained; this occurred during the first day and no subsequent peeling was observed. In two other cases, adherence dropped to about 40%. Six rats kept over 80% of their film surfaces attached.

Of the unburned group of twelve, the films on ten rats had dislodged to the extent of 50% or more by the third day.

Three tentative conclusions may be drawn from the adherence observations:

- Adherence to burned tissue seems better than to unburned.
- PVIPAA films did not adhere as well as gelatin films on the unburned rats.
- The effect of the particular drug on adherence is minimal.

4.3.9 Transdermal Absorption of Radiolabeled Drugs

Results of these experiments are inconclusive. Although radioactivity appeared in both feces and urine, it was not clear by what mechanism it entered the pool of excretion. Three routes are possible:

- transdermal absorption and subsequent metabolism;
- ingestion of film material by rats and subsequent excretion; and
- contamination of urine or feces by film particles.

The range of radioactivity in excreta varied widely among animals with similar treatments. The highest correlation of data was between percent of film lost and label found in urine and feces. Because the total activity applied to each animal was in the order of 10^7 DPM and the amount collected was 10% of this or in many cases, much less, it is clear that transdermal absorbance was low.

Section 5

THE SEARCH FOR AN IMPROVED POLY- ϵ -CAPROLACTONE WIPE-ON SYSTEM

5.1 Fluorocarbons as Solvents for Poly- ϵ -Caprolactone

5.1.1 Background--Fluorocarbons

Fluorocarbon compounds are a family of organic molecules containing one or more carbon atoms and fluorine. Chlorine, bromine, or hydrogen may also be included in the molecule. These molecules have the general characteristics of non-flammability, low toxicity, chemical and thermal stability, high density, low boiling points, low viscosity, and low surface tension. These compounds find use as refrigerants, propellants, solvents, cleaning fluids, chemical intermediates, foam blowing agents, dielectric fluids, and fire extinguishing agents. Fluorocarbons are more commonly known as Freons[®], which are manufactured by E. I. DuPont de Nemours. Other chemical companies also manufacture fluorocarbons.

In the food industry, gaseous blends of nitrous oxide and 1,1,1,2,2-pentafluoro-2-chloroethane ("Freon" 115) are used as propellants for whipped cream. In the cosmetic field, dichlorodifluoromethane ("Freon" 12) and 1,2-dichloro-1,1,2,2-tetrafluoroethane ("Freon" 114) are used as propellants. They cause no undesirable effects when applied topically and are odorless. Some fluorocarbons, such as 1,1,2-trichloro-1,2,2-trifluoroethane ("Freon" 113) are used primarily as solvents.

Because of these properties, preliminary testing, and the fact that a European burn spray (Novitas) uses a fluorocarbon mix as a propellant, these chemicals were investigated as solvents for poly- ϵ -caprolactone (PCL). Samples of suitable solvents were obtained from Mr. Larry Hall, Freon Products Division, E. I. DuPont De Nemours, Delaware.

5.1.2 Fluorocarbons Physical Data

The physical properties of fluorocarbons including boiling point, formula, and molecular weight are listed in Table 5.1.

5.1.3 Experimental Results--Fluorocarbons

The first experiment involved dissolving PCL in "Freon 22" (CHClF_2), which has a boiling point of -40.8°C . Liquid CHClF_2 was poured into a beaker containing shredded PCL. A wispy, fine white material was left in the beaker after the solvent rapidly boiled off. The PCL was stretchy and thin, and seemed to be a good start at using fluorocarbons as solvents for PCL. Different compounds with higher, and thus, more suitable boiling points were investigated.

"Freon 11" (boiling point 23.8°C) was placed in a beaker with PCL. "Freon 11" did not dissolve the polymer at all, although it did wet it. Addition of methylene chloride to the mixture did effect dissolution of the polymer, but only as much as could dissolve in the methylene chloride went into solution. When the mixture was applied to the back of the hand, the resulting film was extremely difficult to remove. The film was very thin and it was hard to determine where it had been applied. "Freon 11" was abandoned as a solvent.

In similar experiments "Freon 113" (b.p. 47.6°C) and "Freon 12" (b.p. -29.8°C) were examined as solvents. "Freon 113" wetted but did not dissolve PCL. "Freon 12" did not wet or dissolve the polymer.

5.1.4 Discussion--Fluorocarbons

Fluorocarbons are, unfortunately, not suitable solvents for poly- ϵ -caprolactone. Freons that were tested in this program did not dissolve the polymer. Also, solvents which we had targeted for investigation because of their suitable boiling points ("Freon 21" and "Freon 112") are no longer manufactured.

Table 5.1

PHYSICAL PROPERTIES OF "FREON" PRODUCTS AND OTHER FLUORINATED COMPOUNDS

PRODUCT	FORMULA	MOLECULAR WEIGHT	BOILING POINT	
			°F	°C
"Freon" 14	CF ₄	88.0	-198.3	-128.0
"Freon" 503	CHF ₃ /CClF ₃	87.3	-127.6	- 88.7
"Freon" 23	CHF ₃	70.0	-115.7	- 82.0
"Freon" 13	CClF ₃	104.5	-114.6	- 81.4
"Freon" 116	CF ₃ -CF ₃	138.0	-108.8	- 78.2
"Freon" 13B1	CBrF ₃	148.9	- 72.0	- 57.8
"Freon" 502	CHClF ₂ /CClF ₂ -CF ₃	111.6	- 49.8	- 45.4
"Freon" 22	CHClF ₂	86.5	- 41.4	- 40.8
"Freon" 115	CClF ₂ -CF ₃	154.5	- 37.7	- 38.7
"Freon" 500	CCl ₂ F ₂ /CH ₃ CHF ₂	99.3	- 28.3	- 33.5
"Freon" 12	CCl ₂ F ₂	120.9	- 21.6	- 29.8
"Freon" 114	CClF ₂ -CClF ₂	170.9	38.8	3.8
"Freon" 21	CHCl ₂ F	102.9	48.1	8.9
"Freon" 11	CCl ₃ F	137.4	74.9	23.8
"Freon" 113	CCl ₂ F-CClF ₂	187.4	117.6	47.6
"Freon" 112	CCl ₂ F-CCl ₂ F	203.9	199.0	92.8
FC 114B2	CBrF ₂ -CBrF ₂	259.9	117.1	47.3
1,1-Difluoroethane*	CH ₃ -CHF ₂	66.1	- 13.0	- 25.0
1,1,1-Chlorodifluoroethane**	CH ₃ -CClF ₂	100.5	14.5	- 9.7
Vinyl Fluoride	CH ₂ =CHF	46.0	- 97.5	- 72.0
Vinylidene Fluoride	CH ₂ =CF ₂	64.0	-122.3	- 85.7
Hexafluoroacetone	CF ₃ COCF ₃	166.0	- 18.4	- 28.0
Hexafluoroisopropanol	(CF ₃) ₂ CHOH	168.1	136.8	58.2

It is worth noting here that the fluorocarbon family is not as non-toxic as was once believed and published in information bulletins. Clayton (1967) reports that trichlorotrifluoroethane, $\text{CCl}_2\text{F}-\text{CClF}_2$ ("Freon 113"), causes alterations of the dermal and adjacent connective tissues when applied topically. Applications of this compound over five days resulted in weight fluctuations and skin damage in rats. Slight liver changes were also evident. Clayton also cites other investigations in which the effect of a number of fluorocarbons on wound healing speed was examined. The skin of rats became irritated, edematous, and inflamed. The healing of wounds and burns was retarded. Thus, fluorocarbons may not be suitable for topical applications to burn wound patients.

5.2 Solubility Parameters

In an earlier contract (Contract No. N00014-73-C-0201) Dynatech investigated a number of compounds as solvents for poly- ϵ -caprolactone. Candidate solvents were chosen according to solubility parameter values. This section reviews the theory of solubility parameters.

The process of dissolving a polymer in a solvent is governed by the free energy equation:

$$\Delta G = \Delta H - T\Delta S, \quad (5.1)$$

where: ΔG = change in free energy;
 ΔH = heat of mixing;
 T = absolute temperature; and
 ΔS = entropy of mixing.

Because the dissolution of a polymer is associated with a large increase in entropy, the magnitude of the heat term, ΔH , determines the sign of the free energy change, ΔG . There have been many theoretical treatments of this term, but the one proposed by Hildebrand is the most useful. For two components it states:

$$\Delta H_M = V_M \phi_1 \phi_2 [(\Delta E_1/V_1)^{1/2} - (\Delta E_2/V_2)^{1/2}]^2, \quad (5.2)$$

where: ΔH_M = overall heat of mixing;
 V_M = volume of the mixture;
 ΔE = energy of vaporization of component 1 or 2 at zero gas pressure;
 V = molar volume of component 1 or 2; and
 ϕ = volume fraction of component 1 or 2.

Rearranging:

$$\frac{\Delta H_M}{V_M \phi_1 \phi_2} = [(\Delta E_1/V_1)^{1/2} - (\Delta E_2/V_2)^{1/2}]^2. \quad (5.3)$$

The term $\Delta E/V$ is the energy of vaporization per unit volume and is known as the "cohesive energy density." Because the heat of mixing per unit volume is equal to the square of the difference between the square roots of the cohesive energy densities of the components, it is convenient to assign the cohesive energy densities a symbol. Let it equal S :

$$S = (\Delta E/V)^{1/2} \quad (5.4)$$

The solubility parameter, S , is generally reported in $(\text{cal/cc})^{1/2}$. Substituting S into equation 2.3:

$$\frac{\Delta H_M}{V_M \phi_1 \phi_2} = (S_1 - S_2)^2. \quad (5.5)$$

This, it is seen that the heat of mixing of two components depends upon the term $(S_1 - S_2)^2$. As the difference between the solubility parameters approaches zero, the heat of mixing decreases. Thus, when $(S_1 - S_2)^2 = 0$, the solution is assured by the entropy factor in Equation 5.1. In other words, as $S_1 \rightarrow S_2$, the heat of mixing approaches zero. Components with equal solubility parameters are therefore miscible. The high entropy change that occurs during solvation of a polymer makes this possible.

Solubility parameters have been determined for solvents and are conveniently listed in tables; a comprehensive table may be found in Brandrup and Immergut (1966). These values have been determined by a variety of methods, including several methods of calculation from physical constants as well as experimental determination. Polymers, however, usually have solubility parameter ranges, and these are experimentally determined. In addition, the solubility parameter range for a polymer depends on whether the solvent is a strong, moderate or poor hydrogen bonder. Thus, polymers may have three different solubility parameters depending on the classes of the solvents.

PCL is not sufficiently soluble in the "Freons", in water or in the lower aliphatic alcohols to warrant their use in a PCL wipe-on coating. These observations are in accord with their solubility parameters, as discussed in Section 5.3.

5.3 Discussion

Finding a non-toxic, non-irritating, non-flammable solvent for poly- ϵ -caprolactone has proven to be more difficult than expected. Neither water nor alcohols, which were proposed, are good solvents for PCL. In retrospect, this was to be expected due to solubility parameter considerations. From previous experiments with PCL, we know that it dissolves in benzene, dioxane, methylene chloride, and THF. These have solubility parameters from 9.1 - 10.0 and are poor (benzene and methylene chloride) or moderate (dioxane and THF) hydrogen bonders. Ethanol, methanol, and water have solubility parameters of 12.7, 14.5, and 23.4, respectively, and are strong hydrogen bonders. Thus, it is not surprising that poly- ϵ -caprolactone, with a solubility parameter of approximately 9.5, does not dissolve in more desirable solvents. Aliphatic fluorocarbons, which the "Freons" we examined are classified as, are poor hydrogen bonders but have solubility parameters between 5.5 and 6.2. It is not surprising that these were not solvents for PCL either. (Freon 22, as mentioned in Section 5.1.3, may be calculated to have a solubility parameter of 6.8 at 21°C, under 9 atm. pressure, as vs. $S = 7.2$ at -40°C, 1 atm., is thus not much more promising.)

Unless methylene chloride and methyl acetate become acceptable solvents, further work with poly- ϵ -caprolactone wipe-on solutions will most likely be unproductive.

Section 6

A MODIFIED PViPAA MATERIAL

PViPAA, by virtue of its pendant amines, may be reacted with other materials than glutaraldehyde. Some types of reactions of interest might be the following:

- a) $\text{PViPAA-NH}_2 + \text{Drug-CHO} \rightarrow \text{PViPAA-N} = \text{C} - \text{Drug}$;
- b) $\text{PViPAA-NH}_2 + \text{Protein/Peptide-NH}_2 + \text{Coupling Agent} \rightarrow$
 $\text{PViPAA-C.A.-Protein/Peptide}$;
- c) $\text{PViPAA-NH}_2 + \text{di-acid chloride} \rightarrow \text{crosslinked PViPAA}$; and
- d) $\text{PViPAA-NH}_2 + \text{polyfunctional aldehyde} \rightarrow \text{crosslinked PViPAA}$.

Of these reactions, a) and related reactions must be investigated for specific drugs; the target action is controlled release of the drug. Reaction b) might be used to incorporate specific bioactive proteins, such as growth factors, monoclonal antibodies, or indicator enzymes onto PViPAA; the PViPAA could then serve as linker with a controllable degradation rate, depending on the coupling agent used. Reaction c) employs further amidic linkages to completely or partially crosslink the PViPAA; by this method, for example, unsaturated and hydrophobic groups, might be incorporated. Lastly, reaction d) could use a low-cost material such as an altered starch as a crosslinker. In this case, the degradation of the crosslinker might control the gel breakdown time.

6.1 Preparation of Fumaryl-Chloride-Precrosslinked PViPAA (PViPAA-F)

Samples of PViPAA, slightly crosslinked with fumaryl chloride (designated PViPAA-F), were prepared as follows. To a solution of 2.00g PViPAA (B 017468-3A) and 10 drops triethylamine in 40 ml methylene chloride in a flask was added dropwise and with stirring 2.00 ml of 0.051% wt/wt fumaryl chloride in methylene chloride solution. Half the product was reserved, and an additional 1.00 ml of the fumaryl chloride solution added

to the remaining PViPAA solution. The PViPAA was precipitated by adding each product solution dropwise to 300 ml of stirred anhydrous ethyl ether, filtered, washed with 50 ml dry ether, and vacuum-dried 18 hours at room temperature. The products, thus crosslinked at levels of fumaryl chloride: PViPAA of 0.5: and 1.0:1000 (wt/wt), (net weights 0.76 and 0.88g) were then tested for gelation properties.

The test for gelation properties was run in the following way: 30 wt% solutions of parent PViPAA (017468-3A), and the two crosslinked materials (018213-A & -B) were prepared in 0.05 M pH 7 phosphate buffer (Fisher) and their pHs measured at 7.89, 7.88, and 7.64, respectively. The crosslinked-PViPAA solutions were very slightly more viscous than the control, and a slightly hazy, as opposed to clear, yellow. Six wells of an ELISA plate were filled with 200 μ l of solution for each of the polymer solutions. Water diluent was added to each (from 0 to 85 μ l), mixed, and then 1.2% wt/vol glutaraldehyde (E.M. grade) solution added (from 100 μ l to 15 μ l) so as to give final polymer level of 20%, and final glutaraldehyde levels of 0.4, 0.2, 0.15, 0.10, 0.08 and 0.06% wt/vol in the wells; the wells were prepared in order of increasing glutaraldehyde, and each well mixed with a wooden applicator stick immediately upon glutaraldehyde addition.

Gelation was almost instantaneous at 0.4% glutaraldehyde, \leq 45 sec @ 0.2%, \leq 1 min 40 sec @ 0.15%, \leq 3 min 15 sec @ 0.10%, and \leq 4 min 45 sec @ 0.08%. For the 0.15% on down, the firmness of the gel formed increased with increasing crosslinkage of PViPAA. For the 0.06% glutaraldehyde wells, the crosslinked PViPAAs formed soft and medium gels, respectively, by 6 minutes; the uncrosslinked-PViPAA took until 8 minutes to become stringy, and did not set further (remained liquid beyond 15 minutes).

Thus, the lowest level of glutaraldehyde at which a gel could be achieved with fumaryl-chloride-pre-crosslinked PViPAA was shown to be $< 0.06\%$, whereas the parent uncrosslinked PViPAA required between 0.06 and 0.08% glutaraldehyde to gel a 20% polymer solution at pH ~ 7.9 ; this result confirms the feasibility of using this method of pre-crosslinking to easily

and controllably enable the use of lesser amounts of glutaraldehyde in the gel formulation.

6.2 Further Gel-Testing of PViPAA-F/Glutaraldehyde (018227)

PViPAA-F (B018220-2) (1:1000 wt:wt fumaryl chloride/PViPAA) was prepared as described in Section 6.1 above. Solutions of 20% wt/wt PViPAA-F in aqueous buffer (pH 7 0.05 M $\text{KH}_2\text{PO}_4/\text{NaOH}$, Fisher) were tested with a range of glutaraldehyde concentrations, to determine the lowest practical and achievable gelling compositions. Thus, to samples of 0.2 g PViPAA-F and 0.7 ml buffer, diluent water and glutaraldehyde were added as in Table 6.1 below.

Table 6.1

GELATION BEHAVIOR OF PViPAA-F/GLUTARALDEHYDE MIXTURES

SAMPLE NUMBER	ADDED DILUENT H_2O (ml)	1% GLUT. (ml)	pH	TIME TO MEDIUM STRINGINESS	TIME TO SOFT GEL	FINAL % GLUTARAL-DEHYDE/TOTAL
018227-1	0	0.10	7.25	0:40	1:30	0.10
018227-2	.020	.080	7.25	1:20	1:50	0.08
018227-3	.040	.060	7.25	1:55	3:20	0.06
018227-4	.050	.050	7.25	8:10	>25 if at all	0.05
018227-5	.060	.040	7.25	v. sl. 8:00, no increase	----	0.04
018227-6	.070	.055	7.25	2:30	7:30	0.54
018227-7	.080	.055	9.19	1:00	1:20	0.53
018227-8	.090	.040	9.47	2:20	6:00	0.39

At pH 7.25, with 20% PViPAA-F solution, ~0.55% glutaraldehyde would appear to be an ultimate lower limit, with 0.60% being considerably quicker acting. However, with a pH increase to 9.5, glutaraldehyde down to 0.40% will gel. (Alternatively, an increase in PViPAA-F to 30%, with a modest increase in pH, would permit gels at glutaraldehyde levels of 0.4 - 0.5%.)

6.3 Preparation of PViPAA-FF and a Free-Radical-Crosslinked Gel

We attempted to use a free-radical polymerization as an alternative method to the use of glutaraldehyde. This necessitated some modification so that a) the PViPAA would possess moieties capable of transmitting the chain reaction, and of becoming part of the chains so formed, and frequent enough to render the PViPAA easily crosslinkable, and b) the medium could react to link the PViPAA chains, since otherwise the crosslinking would be hindered by steric requirements.

To satisfy a), a derivatized PViPAA (designated PViPAA-FF) was made by taking a degraded, partially acid hydrolysed PViPAA, extensively reacting it with fumaryl chloride and recovering the polymer, which now had many pendant or linking groups containing the carbon-carbon double bonds of the fumaryl moiety. Thus, an ordinary PViPAA (prepared as previously described) was dissolved in $\text{MeCl}_2/\text{EtOH}$ solution, and HCl gas bubbled through. A precipitate formed, which was redissolved by warming in EtOH ; the solution was added to Et_2O to precipitate the PViPAA (B017475-1). This product, which was quite acidic (pH ~2 @10% in H_2O), was quite inferior at gelation with glutaraldehyde, forming only a very weak gel at 15% concentration under the same conditions that the parent PViPAA formed a weak gel at 9.5% concentration. However, it now had many more sites for reaction with fumaryl chloride. This product was dissolved as a 10% solution in MeCl_2 and 2% fumaryl chloride, to a total of 1:60 wt/wt fumaryl chloride: PViPAA, added, with tertiary amine present to buffer. The polymer was precipitated into Et_2O and dried, and will be referred to as extensively fumarylated PViPAA, or PViPAA-FF.

To satisfy b), gel-forming reactant mixtures were made, incorporating various amounts of vinylpyrrolidone (VP) and water. The water decreased viscosity and the VP polymerized to form connections between crosslinkable sites on the PViPAA-F chains. Thus, ingredients as shown in Table 6.2 were mixed in order PViPAA-FF, H₂O, VP and H₂O₂, then incubated at 37°C for 1 hr. Gels formed as shown. One should note that the effective initial level of H₂O₂ is only 0.5%, much below the 3% used for skin disinfection. Thus, it seems that more rapid gelatin (<1 hr) would be feasible (see Section 6.4).

Table 6.2
GELATION BEHAVIOR OF PViPAA-FF WITH H₂O₂ AND VP

	Sample 018224-A	Sample 018224-B	Sample 018224-C	Sample 018224-D	Sample 018224-E	Sample 018224-F
30% H ₂ O ₂	0.02 ml	0.02 ml	0.02 ml	0.02 ml	0.02 ml	0.02 ml
PViPAA-F 018222-1	1.00 g	1.00 g	1.00 g	1.00 g	1.00 g	0 g
VP (ml)	0	.25	.50	.75	1.00	1.00
H ₂ O (ml)	1.00	.75	.50	.25	0	0
Gel formed?	No	Weak	Medium	Weak and Syrupy	No	No

6.4 Further Testing of H₂O₂ Cross-Linking of PViPAA-FF

As an extension of Section 6.3, the crosslinking of PViPAA-FF in aqueous 25% vinylpyrrolidone solution was tested as a function of %H₂O₂ used as initiator. Thus, samples of 0.2 g PViPAA-FF (018222-1) dissolved in 1.0 g aqueous VP (25% VP by volume, 28% by weight) were initiated with the following volumes of 30% H₂O₂, see Table 6.3. Sample F had 0.3 g polymer

Table 6.3

LOW LEVEL PVIPAA-FF/%H₂O₂ INITIATOR LOADINGS

	Sample 018228-A	Sample 018228-B	Sample 018228-C	Sample 018228-D	Sample 018228-E	Sample 018228-F
Volume 30% H ₂ O ₂	0.3 ml	0.2 ml	0.1 ml	0.07 ml	0.04 ml	(0.1 ml)

and 1.0 g liquid, as a test of the dependency of gelation on polymer concentration.

The samples were mixed, then incubated at 37°C and observed over time. Gelation was quite slow; however, by 45 minutes gels had formed as follows:

Table 6.4

GELATION OF PVIPAA-FF AS A FUNCTION OF H₂O₂ CONCENTRATION

Incomplete ← GELATION → Complete				
A	B	D	E & C	F
Remained liquid	Barely Stringy	Slightly less strong than <u>E</u>	Soft, weak gels	Firm, completely set

A remained a liquid, as did controls of 25% VP liquid with and without initiator. In all cases, at least a very thin covering of liquid remained unpolymerized, the probable result of radical quenching by indiffused O₂ from the air.

Thus, the feasibility of initiating gelation of a PVIPAA-FF/VP/H₂O solution by concentrations of H₂O₂ from 2.5% down to at least 0.33% has been demonstrated. Still to be tested is the possibility of redox initiation (Fe²⁺/H₂O₂) which would be expected to increase gelation speed.

6.5 Preparation of Slow-Release, Water-Swellable Boluses of Peroxide-Crosslinked PViPAA-FF (018223)

To test the ability of crosslinked PViPAA-FF (extensively fumarylated PViPAA) gels to incorporate fillers and to withstand leaching, a series of boluses were made with the following compositions (Table 6.5):

Table 6.5
BOLUS FORMULATIONS

	Sample 018223-A	Sample 018223-B	Sample 018223-C	Sample 018223-D	Sample 018223-E
Polymer	<div> <div>←</div> <div>PViPAA-FF 018222-1 1.0 g</div> <div>→</div> </div>				PViPAA 017468-3B 1.0 g
VP	0.38 g	0.38 g	0.38 g	0.38 g	0.38 g
Initiator	0.12 g benzoyl peroxide		<div> <div>←</div> <div>0.08 ml H₂O₂</div> <div>→</div> </div>		
Filler	2.5 g CaSO ₄ ·2H ₂ O	None	2.5 g CaSO ₄ ·2H ₂ O	<div> <div>←</div> <div>2.5 g silica -240 mesh</div> <div>→</div> </div>	

The initiator was mixed with the VP, then polymer and filler were worked in. The boluses were kneaded until uniformly mixed, then stored at 37°C. Hardening was observed as shown in Table 6.6. After 4 hours at 37°C, the extent of crosslinking was checked by leaching each bolus in 200 ml water for 3 days. The resultant integrities of the boluses are shown in Table 6.7.

As can be seen in Table 6.7, the concept of crosslinking through the unsaturations of PViPAA-FF, to produce a water-swellable gel, was shown workable, and the relative performance of benzoyl and hydrogen peroxide as initiators was demonstrated. Gels made in this manner are 1) obviously crosslinkable at body temperatures, 2) physically resistant to

Table 6.6

BOLUS HARDENING OBSERVATIONS

TIME ELAPSED	Sample 018223-A	Sample 018223-B	Sample 018223-C	Sample 018223-D	Sample 018223-E
2 hr	Fairly hard	Pliable, partially elastic, partially plastic	Stiff, but slowly elastic	Pliable, plastic	Pliable, plastic
3 1/2 hr	Hard, cannot be bent	Fairly hard, slowly pliable	Hard, very slowly pliable	Fairly hard, slowly pliable	Pliable

Table 6.7

INTEGRITY OF LEACHED BOLUSES

Incomplete ← ——— CROSSLINKING ——— → Complete				
E	A	B	D	C
Completely dispersed	Limp, barely held together	Mildly yellow floating mass easily deformed with prodding	Cloudy leachate slightly less firm than <u>C</u>	Clear surfaced, but firm and rubbery

disintegration, and 3) able to incorporate substantial amounts of fillers or other foreign material, but are still able to gel adequately.

Section 7

OUTSIDE INTEREST IN THE PViPAA WIPE-ON SYSTEM

7.1 Introduction

The concept of a wipe-on wound covering is very appealing to the general public and to professionals in health care. Presumably because of its simplicity, doctors and scientists who see the material seem to very easily focus on adaptation to their own medical applications. This interest has been a real advantage to the program because it has kept the emphasis on rapid development of a broad-usage product.

7.2 Applications of PViPAA in Burn Treatment

We have consulted regularly with Dr. Nicholas O'Connor of Brigham and Women's Hospital in Boston. Dr. O'Connor is a surgeon who specializes in severe burn treatment and collaborates with others in the most advanced methods of burn wound healing and skin replacement. Dr. O'Connor has suggested use of the wipe-on covering as an adjunct in post-skin-graft situations. We have also discussed with him the use of PViPAA film as a cell growth medium for skin tissue transplants. These are potential research areas which would require clinical collaboration.

7.3 Wound Treatment with Wipe-On Covering

Because the wipe-on covering fits wounds so well, it could find broad usage for treatment of many kinds of wounds. Our in vivo tests had been for short terms (3 days maximum) and had been on burns or intact skin. We obtained an evaluation of the wipe-on system in healing promotion on full excision wounds for 8 days. This testing was a protocol established to evaluate long-lasting dressings. The PViPAA wipe-on was judged to be easy to apply to these wounds, but healing was delayed under the dressing. The healing delay was tentatively attributed to the level of glutaraldehyde used in the formulation which was employed. Subsequently, it has been shown that

this level of glutaraldehyde is not at all necessary for film formation. No information on wound condition at intermediate times between application and removal were obtained in this study. The full report is given in Appendix B.

7.4 PViPAA Film as a Part of a Drug Delivery System

The PViPAA Hydrogel can serve as a drug reservoir. While the focus of the present contract was on release of medication to a burn wound, other drug release applications have been suggested. One consideration is simple transdermal drug delivery on intact skin. This concept has not been explored adequately. We have also found interest in using PViPAA films as reservoirs for electrically stimulated transmission of drugs across intact skin. In particular, Dr. Whitney R. Powers, Professor of Anatomy and Health Sciences at Boston University School of Medicine has suggested use of the films in his use of local anaesthetic delivery in treatments of spasticity.

7.5 PViPAA Film as Conductor in Electrical Therapy for Wound Healing

Because of the intimate contact achieved between the gel and a skin surface, PViPAA has attracted interest as a contact electrode material. In particular, Patrick Carley, R.P.T. at Massachusetts Rehabilitation Hospital, in a collaborative project with Stanley Wainapel, M.D. (University Hospital) and Gerald Kassels, M.D. (also at M.R.H.), is examining the utility of crosslinked PViPAA films as electrode materials, in the healing of small diabetic skin ulcers and bedsores, in a protocol which employs microampere DC current to accelerate healing. Advantages in this application include intimate electrical contact with the target area, transparency to allow viewing of the area, and extreme flexibility when adequately moist or plasticized, and are detailed in DIB-116 (Appendix A). For this purpose, electrolytes (or other drugs as desired) may be included as part of the wipe-on medium, without affecting the gelation substantially.

Appendix A

DYNATECH CORPORATION DISCLOSURES OF INVENTION

DYNATECH CORPORATION
DISCLOSURE OF INVENTION*

DIB-101

3/29/83

Descriptive Title of Invention: Wipe-on Gelling Wound Cover

A. What is the problem that has been solved?

The need for a wound covering for first aid that does all of the following:

- 1) Penetrates to all areas of the wound, as does a liquid.
- 2) Subsequently adheres and forms a pliable film which is a barrier to bacteria and fluid loss, and to mechanical abrasion, as does a film.
- 3) Is transparent to allow visual triage at any subsequent time.
- 4) Can release substantial quantities of therapeutic chemicals including bactericidal and bioactive agents at a controlled rate.
- 5) Is easy to store and to apply.
- 6) Is inexpensive for the benefit rendered.

B. Is this problem a newly recognized problem or is it one that people have tried to solve before? If the latter, what is unsatisfactory about the previous solutions?

Wound-covering materials have been continuously evolving in parallel with advances in medicine. Types of wound coverings used at times have included films, sprays, gels, polymer dusts, grafted skins, foams, and tissue-cultured products. (Reference: Assessment of Wound Therapy Systems, Dynatech Report #2165.) Most recent research has concentrated on the need to replace skin (or other tissues) following excision of damaged debris. These presupposed a ready supply of surgical expertise, sterile conditions, immobilization of patients, and the like. In addition, these "skin replacement" materials are often expensive, reflecting the highly biological purity necessary for long-term tissue compatibility. In contrast to these, we perceived a need for an immediate-use wound therapy material, which would incorporate all of the points of (A) above. Such a material is applied as a viscous liquid, and gels to form a crosslinked, adherent, flexible solid. Previous failures to achieve such a system include:

- 1) Bactericidal liquids - leave no protective layer, do not retard water loss.

(See insert B)

*In this instance brevity is not a virtue. Therefore, if an insufficient space is provided for an adequate answer to any of the questions, or if you feel that information beyond that given in any answer is necessary, please use extra pages. If there is any question, or if the form does not seem to fit the particular instance, please contact the Invention Panel.

Question B. (continued)

- 2) **Preformed** foam overlays - do not penetrate into the wound.
- 3) Drying-type films - usually contain irritating solvents, and may be difficult to look through and remove.
- 4) Other gel-type systems - are applied as gels, have poor adherence or are subject to moisture permeation/drying out/washing away.
- 5) Skins - storage and cost problems.
- 6) Moisture impervious films - must be lanced to avoid fluid buildup; some moisture permeation essential for adherence over time.

C. What are the closest existing products or ways of making products?

Materials which form a gel:

- 1) Gelatin-based foam (Neumann, et al.) J. Biomed Mat. Res. 15, 9 (1981) - almost completely unable to retard water loss.
- 2) Pluronic F-127 gel (Schmolka, JBMR 6:571 (1972) - (gels by temperature changes), no resistance to dissolution by body fluids.

(See inset C.)

D. What is the improved product or the improved way of making a product and why does this solve the problem?

The improved method of making this wound covering incorporates the following elements:

- 1) Application as a viscous liquid, allowing penetration of the wound but adherence of a substantially thick film.
- 2) Crosslinking of components of the liquid to form a gel which adheres to the wound intimately, coheres as a film, and is flexible enough to be worn without cracking.
- 3) Storage as two (or more) liquids, or solids, prepared as two liquids and mixed just before application, one component consisting of polymeric substrate, bactericides, bioactive agents, and plasticizers, the other component consisting of the agent which cross-links the polymer chains.

We have found that cross-linking of certain biocompatible polymer solutions may be carried out on wounds at a controllable speed such that mixing and application of this wound cover are completed before gelation takes place, and that the gel presently formed is firm, non-sticky, and possesses all the desirable attributes in (A).

The polymer used maybe copolymer of vinyl pyrrolidone and allylamine, or other alkyl or aromatic amine bearing at least one polymerizable C-C double bond; the crosslinker may be formaldehyde or glutaraldehyde; the plasticizer may be glycerol or numerous other materials; the supporting solvent may be water, lower alcohols, or mixtures thereof; the therapeutic agents may be any of a spectrum of antibiotics, bacteristats, tissue growth factors, and other drugs; the gelling speed may be controlled by the concentrations and pH of various components; and lastly a wetting agent is necessary in small amounts to achieve skin-wetting for which SDS has been found useful.

E. How does this differ from existing products or ways of making products?

- 1) It is not a preformed solid or gel, which cannot conform to and adhere to a wound without air packets being formed.
- 2) It is not a liquid, which dries, or runs off the wound area.

(See insert E)

Question C. (continued)

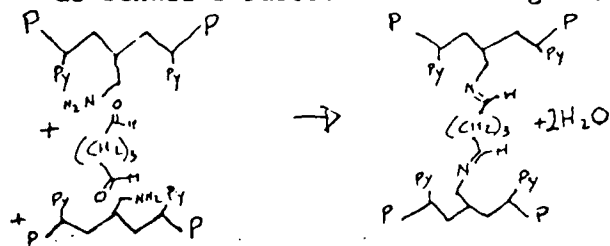
- 3) Hydron [poly(hydroxyethyl methacrylate)] (Nathan, et al.) Trans Am Soc. Artif. Int. Organs 22, 30 (1976) - hard to apply and cracks in use; obscures wounds; often poor adherence.

Materials which are a gel already:

- 1) A plant-based gel, used in a fire blanket (no claims for wound use) Renner-Smith, Pop. Sci. July 1981, 62.
- 2) Second Skin gel pads, essentially non-adherent and only for reducing friction during athletic motions, Physician and Sports Medicine, March 1983, II, (3) "Skin Disorders in Athletes" L.W. Stauffer, (p. 113) 101-119.
- 3) A dextran gel, already prepared as a dressing, Wang and Samji, Abs. Pap. ACS 179, 98 (1980), which would be non-penetrating.

F. What is the best way known to you to make this new product or to carry out the new way of making products? Please attach illustrative drawings if the development is capable of illustration, and full and complete formulae if the development consists of a mixture of ingredients or a way of carrying out a physical or chemical reaction.

The crosslinking reaction which forms the gel is the formation from polymer-chain amines and functionally divalent aldehydes of moieties known as Schiff's bases. Thus a single crosslink would form as follows:



where Py represents pyrrolidone $\left(\begin{array}{c} \text{O} \\ \parallel \\ -\text{N} \end{array} \right)$ and P represents the remainder of the polymer chains.

Additional crosslinks, to form a three-dimensional network, form by the same mechanism at other sites on the P chains.

To make the copolymer of vinyl pyrrolidone and allyl amine, vinyl pyrrolidone and allyl amine in molar proportions of 25:1 are radically polymerized with ϵ -butyl perbenzoate as initiator at 80°C. The reaction product is precipitated into diethyl ether to rid it of allylamine monomer, dried, and ready for use.

Formaldehyde and glutaraldehyde are usable as commercial grade materials, although more purified grades may prove more predictable as crosslinkers.

G. What alternatives are there to the specific things described in paragraph (F) that may be used to achieve substantially the same result?

Crosslinking of polymer chains to form gels may be done by a spectrum of different reactions. For example, aminic residues pendent from polymer chains might be linked by chemical reaction with bifunctional imidate and succinimidyl esters and mixes of these with aridic moieties, difluoro aromatic compounds, p -phenylene di-isothiocyanate, and bifunctional cyclic carbonates, to name a few classes of materials commonly used to crosslink

H. Who first thought of this new product or way of making a product? (See Insert G)

J. Kitchell/S. deRiel

AD-A134 177

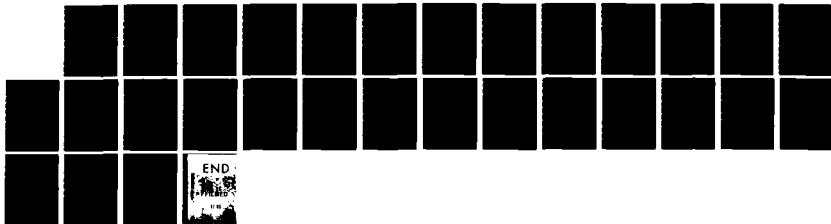
ASSESSMENT OF WOUND THERAPY SYSTEMS(U) DYNATECH R/D CO
CAMBRIDGE MASS S DE RIEL ET AL. 06 OCT 83
DYNATECH-2273 N00014-81-C-0468

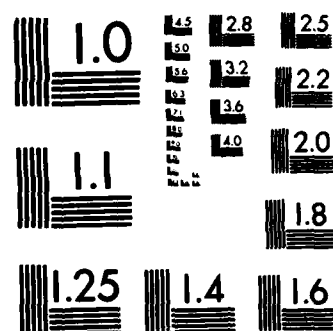
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NL





Question E (continued)

- 3) It does not crack and fissure, thus negating its primary protective function.
- 4) It does not incorporate toxic solvents which further damage or irritate the wound area or whole patient.
- 5) It is not a cloth-based, opaque material which must be removed (exposing the wound anew) for inspection.
- 6) It does not require special skills or equipment to store, mix, and apply.
- 7) It is not prohibitively expensive.
- 8) It is not essentially ineffectual at retarding moisture-loss.

Question G (continued)

lysine amino groups of proteins. Many of these alternative crosslinkers react rapidly (possibly too rapidly) with aminic nitrogens but few, if any, can compete with aldehydes for cost (example: about \$400/gram for many of these "high-tech" linkers; \$6/gram for high purity glutaraldehyde). In addition, several of these are likely to be toxic, which would prove detrimental on an open wound. The reaction of pendant amines with functional dialdehydes to generate Schiff's bases is rather innocuous by comparison.

Another type of reaction leading to a gel ~~could~~ be a free-radical attack on some ~~double~~ bonds incorporated in the polymer chains, or even a total polymerization from monomers (such as might be done with acrylamide). Such free radical reactions, however, are quenched by oxygen and hence would fail in thin films; also monomers are notoriously more toxic than their corresponding polymers, and also tend to be temperature-unstable.

The dialdehydes and amines of the present invention are anticipated to be mildly oxidation sensitive, but moderate storage precautions (e.g. under N_2) could prevent this.

The proposed scheme has the advantage over the complementary reaction between pendant aldehyde groups on polymer chains and crosslinking with an amine or epichlorohydrin in that these crosslinkers might be more damaging to surrounding tissue than the functional dialdehydes, which are immediately immobilized by protein if they diffuse into the wound.

I. Who else made substantial contributions (other than those who merely worked under direction) toward making it work and what did they contribute?

No one.

J. When was it first thought of? On or around 9/3/82.

K. Are there any written records of its inception and development? If so, please identify these records for future reference. SdR notebooks.

#18 (015696-99) 9/3/83

#19 (015751 on) including 1st copolymerization AA & PVP 9/16/82 p 015767

#20 (015951 on) including plasticizer tests

#21 (016401 on)

#22 (016701 on) on film tests

L. Has anyone, other than the persons listed in (H) and (I), followed this development carefully enough to be able to describe details of its progress? This information may be quite necessary should there ever be any future conflict with the patent or patent application of another.

Don Wise; Bob Kispert

M. Has this product or the result of this way of making products:

(a) Ever been described or illustrated in any printed publication? If so, please state when and where, and if possible, attach a copy.

A similar reaction

leading to a different product in Water Soluble Polymers, U.L. Meltzer, Noyes Data Corp. 1972 p. 253-4 and use as "curable coatings; complexing agents

(b) Ever been sold or offered for sale for metals and phenolic compounds".
whom and when. No

(c) Ever been shown or described to persons outside of the company? If so, to whom and when.

Only visitors from ONR Program (and whoever else Don has brought around.)

(d) Ever been used in a proposal? If so, which proposal of what date.

To ONR and subsequent Army proposals.

(e) Been intended for use in a proposal?

(f) Been worked out under a project, the sponsor of which retains rights to inventions?

Yes (Navy).

N. Is this disclosure, to your knowledge, related to any other disclosure submitted previously or simultaneously? No

Date: 3-31-83

Signed:

Date: 5/31/83

*Witnessed and Understood:

Date: 5/31/83

*Witnessed and Understood:

*Persons mentioned in (H) and (I) if possible.

Descriptive Title of Invention:

Biocompatible, amidic-based in-situ-curing staged-polymer matrices.

A. What is the problem that has been solved?

Preparation of a polymeric matrix with all the following properties:

- 1) Controllable hydrophilicity, from moderate to extreme.
- 2) Capacity for both easy covalent attachment of physical adsorption in a protein-like environment of drugs, etc. for release from the matrix.
- 3) Capacity to be fabricated, implanted, etc. in one physical form with subsequent alteration by crosslinking to a form suitable for long-term in vivo uses.
- 4) Controllable in vivo hydrolysis/leaching rates.
- 5) Composition primarily of ^{VP} biocompatible vinyl pyrrolidone, and other, physiologically native, subunits.

(designated hereafter as VP)

B. Is this problem a newly recognized problem or is it one that people have tried to solve before? If the latter, what is unsatisfactory about the previous solutions?

It is a long-standing problem to produce materials both acceptable to the body and capable of accomplishing their tasks, be they structural or therapeutic. Particularly difficult has been finding hydrophilic materials that are not ultimately rejected or irritating. Previous solutions to these problems have used materials which have been fabricated (as by polymerization) and treated (as by leaching) for exorbitant amounts of time to leach out impurities. This is intensely inefficient and requires the use to ~~fit~~ physically conform to the fabricated device ~~what has been fabricated~~, i.e., requires the hand to fit the glove, so to speak. In addition, therapeutic release of bioactive, fragile materials [one class of therapeutic agents (designated hereafter as TA's)] is precluded by the purification times involved.

On the other hand, in situ generation of polymers, as is done ~~with~~ from methyl methacrylate in hip replacements, releases so much heat as to utterly destroy any incorporated biological materials, for example.

(Continued on separate page)

*In this instance brevity is not a virtue. Therefore, if an insufficient space is provided for an adequate answer to any of the questions, or if you feel that information beyond that given in any answer is necessary, please use extra pages. If there is any question, or if the form does not seem to fit the particular instance, please contact the Invention Panel.

Question B. continued.

Thus, creation of final polymeric matrices from medium length, partially polymerized ("staged") prepolymers [designated hereafter "SP's"] is logical, but unpursued up to now, due usually to the toxicity of even the prepolymers.

Question C. continued.

- 3) short chain nylons, which possess outstanding mechanical properties, and are similarly amidic-based, but are somewhat hydrophobic and not suitable for polymerization in vivo.

add: (See also question E.)

Question E. continued.

- 1) being a structurally primarily amidic, due to internal pyrrolidone rings and amidic cross-links, rather than partially poly(esteric), as is bone putty (This will have notable effects on polarity and hydrolysis rates);
- 2) having a controllable number of attachment sites (methylene amines, or their derivatives) for drug, etc. attachment, rather than none, as in the bone putty;
- 3) using a pre-manufacture cross-linker of selectable hydrophobicity, to control the hydrophilicity of the final polymer. This enables a special affinity-type control of therapeutic-agent release, rather than a strictly diffusion or erosion-limited one; and
- 4) being capable of manufacture in water-swellable, gelatinous final forms as well as rigid ones.

add to C.:

→ add to E.): (See also question C.)

This product also derives in general composition from burn/wound covering materials, of composition VP plus aminic material plus glutaraldehyde as cross-linker, developed at Dynatech under Navy contract #

Question B. continued.

Thus, creation of final polymeric matrices from medium length, partially polymerized ("staged") prepolymers [designated hereafter "SP's"] is logical, but unpursued up to now, due usually to the toxicity of even the prepolymers.

Question C. continued.

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Question E. continued.

- 1) being a structurally primarily amidic, due to internal pyrrolidone rings and amidic cross-links, rather than partially poly(esteric), as is bone putty (This will have notable effects on polarity and hydrolysis rates);
- 2) having a controllable number of attachment sites (methylene amines, or their derivatives) for drug, etc. attachment, rather than none, as in the bone putty;
- 3) using a pre-manufacture cross-linker of selectable hydrophobicity, to control the hydrophilicity of the final polymer. This enables a special affinity-type control of therapeutic-agent release, rather than a strictly diffusion or erosion-limited one; and
- 4) being capable of manufacture in water-swellaable, gelatinous final forms as well as rigid ones.

add: developed under contract with the U.S. Army
(DAMD 17-80-C-0186),

C. What are the closest existing products or ways of making products?

Close existing products include:

- 1) epoxy-type resins, which crosslink (often even at body temperatures) but incorporate toxic components and accelerants;
- 2) Dynatech's 'bone putty', which polymerizes VP throughout and onto a poly(propylene fumarate) structural base; and (cont. on separate page)

D. What is the improved product or the improved way of making a product and why does this solve the problem?

The new improved system avoids bioincompatible monomers by using as constituents VP; of known low irritation, an aminic material, and a dicarboxylic acid, the latter two of which have many biocompatible representatives. It avoids excess monomer by using premanufactured, purified "SP" material, which is, however, easily shapable by virtue of molecular weight and low crosslinking. It then avoids excess heat release by additional, controllable in situ crosslinking to set the product, to final values of such important properties as hydrophilicity, biodegradation rate, therapeutic-product release rate, swellability, etc.

This enables the user to:

- 1) manipulate the material in a form very amenable to mixing, and shaping;
- 2) incorporate either by mixing or by known gentle covalent attachment reactions, a wide array of TA's;
- 3) apply the material, with assurance that it will transform (if desired) to a more highly crosslinked state, with optimal in-use properties.

add: ~~This present~~ This improved method of crosslinking enables the formation of ~~more~~ more chemically stable crosslinks than ~~the~~ are formed in the previous method of (VP + amine) copolymer plus ~~glutaraldehyde~~ a dialdehyde (glutaraldehyde); ~~which~~ ^{this} may be advantageous in many applications.

E. How does this differ from existing products or ways of making products?

This product bears a certain resemblance to Dynatech's 'bone putty' material, in both manufacture and use. However, the present material differs by:

(Continued on separate page)

I. Who else made substantial contributions (other than those who merely worked under direction) toward making it work and what did they contribute?

No one.

J. When was it first thought of? 5/26/83

K. Are there any written records of its inception and development? If so, please identify these records for future reference.

Ref. SdR Lab. notebook #24, pp. B 017486-9; also description paper of 5/26/83 in SdR's ONR-4 file.

L. Has anyone, other than the persons listed in (H) and (I), followed this development carefully enough to be able to describe details of its progress? This information may be quite necessary should there ever be any future conflict with the patent or patent application of another.

Don Wise has followed certain details of this development, as has Julie Kitchell.

M. Has this product or the result of this way of making products:

(a) Ever been described or illustrated in any printed publication? If so, please state when and where, and if possible, attach a copy.

Not to my knowledge.

(b) Ever been sold or offered for sale to anyone? If so, please tell to whom and when.

No.

(c) Ever been shown or described to persons outside of the company? If so, to whom and when.

No.

(d) Ever been used in a proposal? If so, which proposal of what date.

No.

(e) Been intended for use in a proposal? No.

(f) Been worked out under a project, the sponsor of which retains rights to inventions? This work related to aspects of ONR-4, ~~but has not been billed to ONR-4.~~

N. Is this disclosure, to your knowledge, related to any other disclosure submitted previously or simultaneously?

~~new date~~ → This is an extension & revision of DIB-114, and related to DIB-101

Date: 6/2/83 Signed: Stanton Robert de Riel

Date: _____ *Witnessed and Understood: _____

Date: _____ *Witnessed and Understood: _____

*Persons mentioned in (H) and (I) if possible.

F. What is the best way known to you to make this new product or to carry out the new way of making products? Please attach illustrative drawings if the development is capable of illustration, and full and complete formulae if the development consists of a mixture of ingredients or a way of carrying out a physical or chemical reaction.

Steps in manufacture of the polymeric final product:

- I. Preparation of the initial PViPAA [poly(vinyl pyrrolidone/allylamine)] material:

(letting AA designate "allylamine")

VP + AA + free radical initiator \rightarrow PViPAA. Thus, 1 mol VP, + $\geq .03$ mol allylamine (or other vinyl-type/primary-aminic/material) + $\geq .01$ mol t-butyl perbenzoate \rightarrow (EtOH soln. $\geq 80^\circ\text{C}$, 16 hrs) \rightarrow PViPAA.

Final composition and molecular weight are controlled by concentrations of reactants. The PViPAA is purified, by precipitation into diethyl ether, and the precipitate dried.

- II. Reaction with initial cross-linker.

PViPAA + alkene dioyl chloride (in MeCl_2 soln.) \rightarrow PViPAA/SP. Thus, to 1g PViPAA (molecular weight ~ 3000) containing q.s. tertiary amine (e.g. triethylamine) to accept the HCl formed, in MeCl_2 soln.) add dropwise $\geq .001\text{g}$ fumaroyl (di)chloride (also in MeCl_2 Soln.) \rightarrow PViPAA/SP. After adding EtOH to destroy any residual unreacted fumaroyl chloride, this PViPAA/SP may be slurried and reprecipitated into Et_2O .

- III. Covalent attachment to PViPAA/SP of therapeutic agent - optional step. If desired, at this point therapeutic agent (TA) may be attached to residual amines on a portion, or all, of the PViPAA/SP, using a gentle difunctional coupling agent directly (where applicable), having first further derivatized the polymeric amines or the TA functionalities as necessary.

- IV. Mixing for application.

The PViPAA/SP/(TA) of steps II/III, additional TA(s), plasticizers, release agents, solvents, fillers, and the like, a polymerization initiator (such as benzoyl peroxide), and (optionally) a polymerizable filler (such as VP) are thoroughly mixed, then formed (as necessary) to final shape. The mixture is then applied as desired (as an implant, a coating, etc.) and it proceeds to cure to final form in situ, using body heat to initiate the peroxide decomposition.

G. What alternatives are there to the specific things described in paragraph (F) that may be used to achieve substantially the same result?

As alternatives, any other methods of:

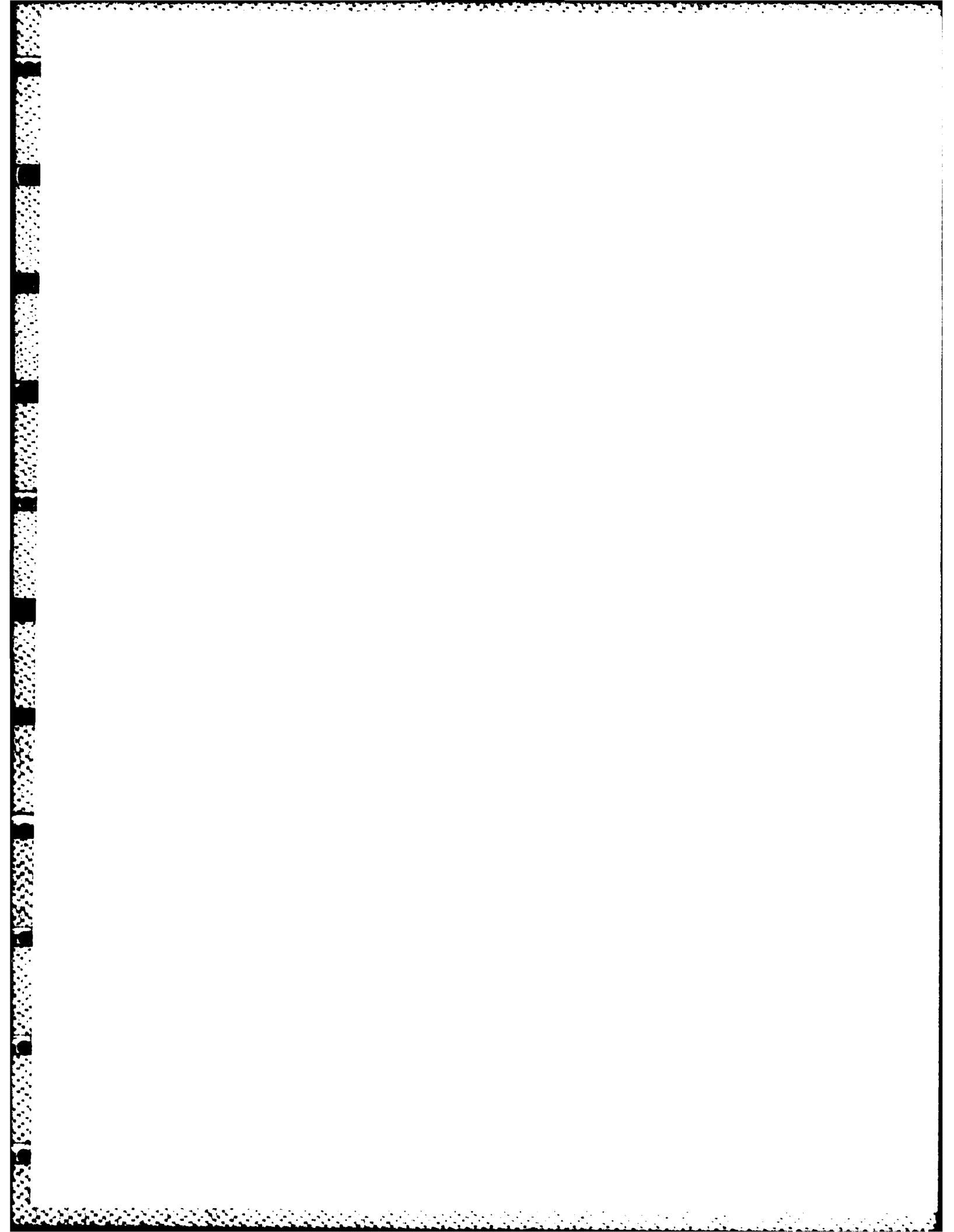
- 1) creating reactive sites on a PVP chain, either during polymerization (by incorporation of monomers with pendant reactive groups), or by attack on a prepared PVP material to hydrolyse some of the pyrrolidone amide rings, or by attack on other points on the pyrrolidone rings, or

- H. Who first thought of this new product or way of making a product? (continued on separate page)

Stanton de Riel

Question G. continued.

- 2) utilizing some or all these sites for attachments of TA's or cross-linker materials, or
- 3) incorporation into a "SP" material of C=C bonds, or other free-radical polymerizable sites, in any other moiety than a difunctional cross-linker, e.g., as a monofunctional material (an alkenoyl (mono)chloride) reacted covalently with the PViPAA, or by generation of a C=C bond in a susceptible site by a dehydrogenation or other elimination reaction, would be essentially alternative ways of generating equivalent pre-polymerized "SP" materials suitable for in situ curing. However, copolymerization of a PViPAA, then cross-linking with an alkendioyl dichloride, is conceived as being very cost-effective and foolproof.



DYNATECH CORPORATION
DISCLOSURE OF INVENTION

Title of Invention:

Date: June 29, 1983

Electrically conductive gels applied as liquids.

A. What is the problem that has been solved?

Obtaining and maintaining intimate electrical contact with all parts of a surface (animate or inanimate) of complex shape.

B. Is this problem a newly recognized problem or it is one that people have tried to solve before? If the latter, what is unsatisfactory about the previous solutions?

This is an old problem. For non-hydrous inanimate objects which can withstand vacuum, sputtering with a metal film affords an electrical contact. Other techniques include contact by liquids (mercury, saline, etc.), paste and gels (applied as gels), and pads of saline-saturated foam, carbon-impregnated plastic, and the like. These methods suffer from various specific and general defects, including rigidity (inability to conform to all parts of the target surface), reactivity with the target surface, opacity (thus preventing trans-device inspections), fluidity (inability to hold an electrode down without additional securing) or impermeability (preventing diffusion of desired substances in either direction through the layer).

C. What are the closest existing products or processes?

With particular application to the biomedical sphere, electrode-contacts to skin include Ag/AgCl-paste electrodes in vinyl foam (mfr: Healthco) (for potential measurements), sticky carbon-impregnated pads (commonly used for transcutaneous electrical nerve stimulation, TENS), conductive gels (for passage of large currents), and pre-gelled, saline impregnated pads of hydrogel (second skinTM) (recommended as primary dressings only).

D. What is the improved product, process, or the improved way of making a product? Why does this solve the problem?

The improved product is a conductive material, applied to the target surface as a liquid (in which an electrode can be embedded); this liquid material then gels to a firm, durable, flexible film. As the liquid, the product is capable of conforming and adhering to a surface of arbitrary size and shape; as the flexible film, it is capable of spontaneous, unassisted adherence, while maintaining flexibility, transparency, durability, conductivity, and controllable permeability.

Within moderate limits, physical properties such as specific gravity and rigidity, and optical properties such as transparency to various wavelengths of electromagnetic radiation, can be controlled. Thus the gel might be alternatively or simultaneously used for controlled transmission of:

- 1) sound: inspections such as echocardiography, and ultrasound imaging on humans or other objects.
- 2) light: for therapeutic stimulation of a biological area, or bombardment of a sample, while applying/sensing current; alternatively, for blocking light or other EM radiation, either for therapeutic or for position-indicating purposes.
- 3) vibrations: for decoupling the motion of an object from a sensor, while retaining electrical contact -- useful for delicate sensors in rugged conditions.

E. How does this differ from existing products or ways of making products?

No existing products that we are aware of combine the advantages of application as a liquid, the irreversible gelation by chemical process, and the in-use properties as an adherent gel-film.

- F. What is the best way known to you to make this new product or to carry out the new way of making products? Please attach illustrative drawings if the development is capable of illustration, and full and complete formulae if the development consists of a mixture of ingredients or a way of carrying out a physical or chemical reaction.

Many materials are physically and chemically capable of participating in a chemical cross-linking reaction to form such a gel film. The one we have found most useful from the standpoints of biocompatibility, gentleness and controllability of the cross-linking reaction, and non-digestibility bacterially we term "PViPAA", for poly(vinyl pyrrolidone/allyl amine), a co-polymer of VP (vinyl pyrrolidone) and AA (allyl amine) [for preparation, see DIB 101]. An aqueous solution of this, containing electrolyte, is mixed with a solution of glutaraldehyde, a crosslinker (as given in DIB 101); the resultant liquid is spread on the target object, and gels after a time controllable by pH, concentrations of reactants, etc. After gelation, the gel is stable to pHs in the range of 1 to 13; gels form unaffected by the presence of substantial electrolyte concentrations (up to at least 14% wt/vol. NaCl). Additional materials may be incorporated in the gels by inclusion in the source solutions, or subsequent application to the gel, such as wetting agents, plasticizers, bioactive peptides and proteins, antibiotics, etc.

- G. What alternatives are there to the specific things described in paragraph (F) that may be used to achieve substantially the same result?

The specific cross-linking reaction used may be accomplished with any pairs of ingredients offering A) free primary amine groups, and B) dialdehydes. Other cross-linking reactions suffer from various drawbacks, as discussed in DIB 101; certain modifications to the product/process may be of use, as discussed in DIB 114.

- H. Who first thought of this new product or way of making a product?

Stanton de Riel

- I. Who else made substantial contributions (other than those who merely worked under direction) toward making it work and what did they contribute?

No one.

- J. When was it first thought of?

Around 3/1/83.

- K. Are there any written records of its inception and development? If so, please identify these records for future reference. List Research Notebook pages pertinent.

Internal Dynatech position papers, dated 3/1/83, in SdR's "Gel Electrodes" file folder. Concept put on "hold" at that time due to apparent lack of use of electric stimulation to heal any other tissue than bone. Since P. Carley (see M.(C) below) is investigating such a use, this product is now timely.

- L. Has anyone, other than the persons listed in (H) and (I), followed this development carefully enough to be able to describe details of its progress? This information may be quite necessary should there ever be any future conflict with the patent or patent application of another.

Julie Kitchell

Donald Wise

Ralph Wentworth

- M. Has this product or the result of this way of making products:

- (a) Ever been described or illustrated in any printed publication? If so, please state when and where, and if possible, attach a copy.

No.

- (b) Ever been sold or offered for sale to anyone? If so, please tell to whom and when.

No.

- (c) Ever been shown or described to persons outside of the company? If so, to whom and when.

Concept discussed 6/23/83 with Patrick Carley of Massachusetts Rehabilitation Hospital Physical Therapy Department.

- (d) Ever been used in a proposal? If so, which proposal of what date.

No.

- (e) Been intended for use in a proposal?

No.

- (f) Been worked out under a project, the sponsor of which retains rights to inventions?

Most of the work on this carried out on ONR-3 and ONR-4 projects.

- N. Is this disclosure, to your knowledge, related to any other disclosure submitted previously or simultaneously?

DIB 101, DIB 114.

Date: 7/6/1983 Signed: Stanton Robert de Riel
Date: 7/11/83 *Witnessed and Understood: Julie Kitchell
Date: 1/13/83 *Witnessed and Understood: Donald Wise

* Persons mentioned in (H) and (I) if possible.

DYNATECH CORPORATION
DISCLOSURE OF INVENTION*

DIB-118

Descriptive Title of Invention:

Extension of utility of "wipe-on" burn wound covering to general combat casualty management situations (ex. shrapnel wounds)

A. What is the problem that has been solved?

Feasibility of a rapid film-forming hydrogel for immediate post-burn treatment has been demonstrated. Application to general combat casualties other than burns, such as shrapnel wounds, is presented as an independent and novel invention.

In vivo testing using miniature swine and rats has been useful in developing the immediate post-burn wound covering and these animals should serve as useful models for evaluating more general combat casualty situations. Of special interest will be identifying problems of bleeding and body fluid interfering with rapid cross-linking of the polymeric film.

It is anticipated that modifications in the polymeric film formulation will be required in order to be used for more general combat casualties other than the special burn wound case, such as shrapnel wounds.

B. Is this problem a newly recognized problem or is it one that people have tried to solve before? If the latter, what is unsatisfactory about the previous solutions?

Scientists and engineers at Dynatech have developed, under funding from the Office of Naval Research and technical direction from the U.S. Naval Medical Research and Development Command, an immediate post-burn wound covering material. Briefly, the objective is to apply via a "wipe-on" type application, a viscous water-based fluid. By the nature of the components of this viscous fluid, within about 15-30 seconds after application it forms (cross-links) into a flexible polymeric film. This "wipe-on" type viscous fluid is to be applied immediately after a burn wound occurs. The polymeric film so formed has a pervaporation rate equivalent to that of human skin. Thus, the immediate post-burn application significantly reduces water loss and maintains a clean wound. Further, the polymer film may contain an antibiotic or bactericide such that controlled release of this active agent into the burn wound occurs.

*In this instance brevity is not a virtue. Therefore, if an insufficient space is provided for an adequate answer to any of the questions, or if you feel that information beyond that given in any answer is necessary, please use extra pages. If there is any question, or if the form does not seem to fit the particular instance, please contact the Invention Panel.

C. What are the closest existing products or ways of making products?

See D below.

D. What is the improved product or the improved way of making a product and why does this solve the problem?

It is of interest to note that while much development work has taken place to develop practical burn wound coverings, essentially all this emphasis has been related to post-excision coverings. This includes development work on a number of synthetic coverings, largely to replace the use of porcine and cadaver skins. Post-excision or post-debridement coverings are applied at a field hospital or trauma burn center within 24-48 hours after the burn occurs. Essentially no other work other than that done at Dynatech has been directed to provide an immediate post-burn covering. The objective of the immediate post-burn wound covering is to provide immediate in-field care with-in the 24-48 hour period until the burned patient is evacuated to a hospital where excision of eschar, application of standard post-excision covering, and long term recovery takes place.

E. How does this differ from existing products or ways of making products?

F. What is the best way known to you to make this new product or to carry out the new way of making products? Please attach illustrative drawings if the development is capable of illustration, and full and complete formulae if the development consists of a mixture of ingredients or a way of carrying out a physical or chemical reaction.

See Final Report on ONR Contract No. N00014-81-C-0468 dealing with immediate post-burn "wipe-on" type burn covering. This present invention applies this earlier work to general combat casualty situations such as shrapnel wounds.

G. What alternatives are there to the specific things described in paragraph (F) that may be used to achieve substantially the same result?

None.

H. Who first thought of this new product or way of making a product?

D.L. Wise

I. Who else made substantial contributions (other than those who merely worked under direction) toward making it work and what did they contribute?

J.P. Kitchell

S.R. de Riel

J. When was it first thought of? January 1983.

K. Are there any written records of its inception and development? If so, please identify these records for future reference.

Letter to USAMRDC of January 5, 1983 by D.L. Wise.

L. Has anyone, other than the persons listed in (H) and (I), followed this development carefully enough to be able to describe details of its progress? This information may be quite necessary should there ever be any future conflict with the patent or patent application of another.

J.P. Kitchell

M. Has this product or the result of this way of making products:

(a) Ever been described or illustrated in any printed publication? If so, please state when and where, and if possible, attach a copy.

No

(b) Ever been sold or offered for sale to anyone? If so, please tell to whom and when.

No.

(c) Ever been shown or described to persons outside of the company? If so, to whom and when.

See January 5, 1983 letter of D.L. Wise to USAMRDC.

(d) Ever been used in a proposal? If so, which proposal of what date.

See January 5, 1983 letter of D.L. Wise to USAMRDC.

(e) Been intended for use in a proposal?

See January 5, 1983 letter of D.L. Wise to USAMRDC.

(f) Been worked out under a project, the sponsor of which retains rights to inventions?

No.

N. Is this disclosure, to your knowledge, related to any other disclosure submitted previously or simultaneously?

Date: 9/21/83 No.

Signed:

Daniel L. Wise

Date: 9/29/83

*Witnessed and Understood:

J.P. Kitchell

Date: 9/29/83

*Witnessed and Understood:

Stanton de Riel

*Persons mentioned in (H) and (I) if possible.

DYNATECH CORPORATION
DISCLOSURE OF INVENTION*

DIB-119

Descriptive Title of Invention:

Hydrogel Transdermal Controlled Release Drug Delivery System

A. What is the problem that has been solved?

Development of a hydrogel matrix system with the following properties:

1. Biocompatible
2. Adhesive to epidermal tissue
3. Applicable as a liquid or as a preformed gel
4. Drug compounds may be incorporated in the matrix before or after gel formation
5. Drug release may be maintained for several days.

B. Is this problem a newly recognized problem or is it one that people have tried to solve before? If the latter, what is unsatisfactory about the previous solutions?

Transdermal drug delivery has increased since the appearance of three products on the market: Nitrodur and Trans derm-nitro, which release nitroglycerine; and Tranderm-V which releases scopolamine.

Problems which are encountered include: skin irritation, incomplete delivery, limited length of delivery, and the complexity of the device.

*In this instance brevity is not a virtue. Therefore, if an insufficient space is provided for an adequate answer to any of the questions, or if you feel that information beyond that given in any answer is necessary, please use extra pages. If there is any question, or if the form does not seem to fit the particular instance, please contact the Invention Panel.

C. What are the closest existing products or ways of making products?

Membrane permeation controlled devices: Transderm-V and Transderm-nitro; matrix-diffusion-controlled devices: Nitro-dur; microsealed drug delivery devices: Nitro disc.

D. What is the improved product or the improved way of making a product and why does this solve the problem?

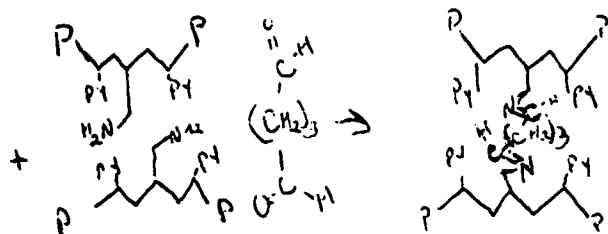
The product is non-irritating, even on wounded tissue, it has a high drug loading potential, and it is a simple device. In its simplest form, the product would consist only of the drug-loaded gel.

E. How does this differ from existing products or ways of making products?

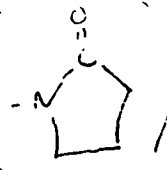
The product is a new polymer, the matrix may be varied by extent of crosslinking, it may be cast in place, it can absorb drug after casting.

F. What is the best way known to you to make this new product or to carry out the new way of making products? Please attach illustrative drawings if the development is capable of illustration, and full and complete formulae if the development consists of a mixture of ingredients or a way of carrying out a physical or chemical reaction.

The crosslinking reaction which forms the gel is the formation from polymer-chain amines and functionally divalent aldehydes of moieties known as Schiff's bases. Thus a single crosslink would form as follows:



Where Py represents pyrrolidone and P represents the remainder of the polymer chains.



Additional crosslinks, to form a three-dimensional network, form by the same mechanism at other sites on the P chains.

To make the copolymer of vinyl pyrrolidone and allyl amine, vinyl pyrrolidone and allyl amine in molar proportions of 25:1 are radical-polymerized with *n*-butyl perbenzoate as initiator at 80°C. The reaction product is precipitated into diethyl ether to rid it of allylamine monomer, dried, and ready for use.

Formaldehyde and glutaraldehyde are usable as commercial grade materials, although more purified grades may prove more predictable as crosslinkers.

The drug may be dissolved or suspended in the polymer solution prior to gel formation, or it may be added as a liquid after gel formation.

G. What alternatives are there to the specific things described in paragraph (F) that may be used to achieve substantially the same result?

Crosslinking of polymer chains to form gels may be done by a spectrum of different reactions. For example, aminic residues pendent from polymer chains might be linked by chemical reaction with bifunctional imidate and succinimidyl esters and mixes of these with azidic moieties, difluoro aromatic compounds, *p*-phenylene di-isothiocyanate, and bifunctional cyclic carbonates, to name a few classes of materials commonly used to crosslink (See Insert G)

H. Who first thought of this new product or way of making a product?

J.P. Kitchell/S.R. deRiel

I. Who else made substantial contributions (other than those who merely worked under direction) toward making it work and what did they contribute?

None.

J. When was it first thought of?
September 1982

K. Are there any written records of its inception and development? If so, please identify these records for future reference.

Yes - attached.

L. Has anyone, other than the persons listed in (H) and (I), followed this development carefully enough to be able to describe details of its progress? This information may be quite necessary should there ever be any future conflict with the patent or patent application of another.

Donald L. Wise

M. Has this product or the result of this way of making products:

(a) Ever been described or illustrated in any printed publication? If so, please state when and where, and if possible, attach a copy.

No.

(b) Ever been sold or offered for sale to anyone? If so, please tell to whom and when.

No.

(c) Ever been shown or described to persons outside of the company? If so, to whom and when.

Yes - ONR Reports

(d) Ever been used in a proposal? If so, which proposal of what date.

To USAMRDC, PBD-180A

(e) Been intended for use in a proposal?

Yes.

(f) Been worked out under a project, the sponsor of which retains rights to inventions?

Yes, ONR-4

N. Is this disclosure, to your knowledge, related to any other disclosure submitted previously or simultaneously?

Yes - DIB-101
Date: 9/22/83 Signed: Stanley Robert de Riel
Date: 9/22/83 *Witnessed and Understood: W. J. Kitchell
Date: 9/22/83 *Witnessed and Understood: Robert J. Lumb

*Persons mentioned in (H) and (I) if possible.

Question G (continued)

lysine amino groups of proteins. Many of these alternative crosslinkers react rapidly (possibly too rapidly) with aminic nitrogens but few, if any, can compete with aldehydes for cost (example: about \$400/gram for many of these "high-tech" linkers; \$6/gram for high purity glutaraldehyde). In addition, several of these are likely to be toxic, which would prove detrimental on an open wound. The reaction of pendant amines with functional dialdehydes to generate Schiff's bases is rather innocuous by comparison.

Another type of reaction leading to a gel could be a free-radical attack on some double bonds incorporated in the polymer chains, or even a total polymerization from monomers (such as might be done with acrylamide). Such free radical reactions, however, are quenched by oxygen and hence would fail in thin films; also monomers are notoriously more toxic than their corresponding polymers, and also tend to be temperature-unstable.

The dialdehydes and amines of the present invention are anticipated to be mildly oxidation sensitive, but moderate storage precautions (e.g. under N_2) could prevent this.

The proposed scheme has the advantage over the complementary reaction between pendant aldehyde groups on polymer chains and crosslinking with an amine or apichlorohydrin in that these crosslinkers might be more damaging to surrounding tissue than the functional dialdehydes, which are immediately immobilized by protein if they diffuse into the wound.

Question K (continued)

References to Drug Release from PViPAA Films
for Disclosure Preparation

- #23 016951 on, including enzyme incorporation (alk. phos.) 016988 and initiator 016992
- #24 017460 on, including
- 1) pH adjustment before precipitation 017473
 - 2) gelation speed as f(pH, % glut) 017477
 - 3) [Sulfadiazine Release] (from gelatin films) 017492
 - 4) PViPAA/fumaryl chloride copolymers 017486
- #25 017651 on, including
- 1) leaching and binding of BSA to gel films 017659, 672
 - 2) use of PEG's as protein insolubilizers 017665, 690
 - 3) incorporation of electrolytes during casting 017673
 - 4) testing pH stability of gel 017673
 - 5) sending samples to P. Carley for D.C. wound healing use 017679
- #26 017953 on, including
- 1) physical properties of PViPAA/PEG films 017961
 - 2) leaching Sulfadiazine from PViPAA films 017972
 - 3) leaching Na Penicillin G from PViPAA films 017981
 - 4) leaching BSA/PLGA from PViPAA films 017996
 - 5) sending samples to D. Rovee @ J&J for animal tests 017966
- #27 018201 on, including precision gelation testing of fumaryl chloride-X-linked PViPAA 018208

Appendix B

DYNATECH BURN DRESSING EFFECTS ON WOUND REPAIR

September 14, 1983

SUBJECT: DYNATECH Burn Dressing effects on wound repair

SUMMARY

An experimental formed-in-place dressing from DYNATECH R/D Company was applied to full-thickness excisions on guinea pigs for eight days beneath a gauze dressing or beneath a semi-occlusive BIOCLUSIVE Transparent Film Dressing. Under both conditions, the DYNATECH Dressing severely delayed repair.

OBJECTIVE

Our goal was to observe the ease of application, pliability and wound-tissue interactions of a normally repairing full-thickness wound with DYNATECH Dressing, and to see if it acted as an occlusive dressing.

METHOD

Circular 4 square centimeter standard full-thickness excisions were made mid-dorsally on 24 adult male Hartley Strain guinea pigs. The area of each wound was immediately measured using an OPTOMAX System III Image Analyzer.

Six wounds in each of 4 groups were dressed as follows:

1. BIOCLUSIVE Transparent Film Dressing (Johnson & Johnson Products, Inc., New Brunswick, New Jersey)
2. DYNATECH Dressing beneath BIOCLUSIVE Transparent Film Dressing (DYNATECH OCC)
3. one 4-ply gauze sponge (AIR EXPOSED)
4. DYNATECH Dressing covered with one 4-ply gauze sponge (DYNATECH A E)

All dressings were held in place with elastic tape during the next 8 days after wounding.

Ease of application, time for the DYNATECH to set up, pliability of the dressing and mechanical damage to the dressing during use or to the underlying tissue upon removal were observed.

Wound areas were again measured on the eighth day after wounding, and wound repair was quantified as percent contraction = $(\text{Day 0 area} - \text{Day 8 area}) / (\text{Day 0 area})$.

RESULTS

1. Ease of application

The DYNATECH Dressing was easy to apply and set up in less than 15 minutes.

2. Pliability

It was solubilized beneath the occlusive dressing and dried to become a composite with the dried wound eschar beneath the gauze sponge. No mechanical damage to the dressing was observed during the 8-day dressing period.

3. Tissue damage

Both the DYNATECH-Treated wounds covered with BIOCLUSIVE Transparent Film Dressing and those covered with gauze sponges suffered severely delayed contraction relative to their respective control wounds. This may be due to some toxic chemical, such as glutaraldehyde, leaching out of the DYNATECH Dressing. There was considerable inflammation surrounding the wounds dressed with DYNATECH beneath the BIOCLUSIVE Transparent Film Dressing. This inflammation appeared in areas contacting the solubilized DYNATECH.

4. Prevention of dehydration

The gauze-covered DYNATECH Dressings permitted formation of a dried eschar between the dressing and the wound. This eschar remained attached to the DYNATECH Dressing upon removal. The fact that an eschar formed indicates that the DYNATECH Dressing did not prevent dehydration of the wound.

Conclusions

The DYNATECH Dressing formulation investigated in this experiment severely retarded full-thickness wound repair and was irritating to intact skin surrounding the wounds. It also acted as a non-occlusive dressing, permitting the wound surface to dry out and become incorporated into the dressing. Based on these results, this formulation would not be recommended for use on repairing wounds.

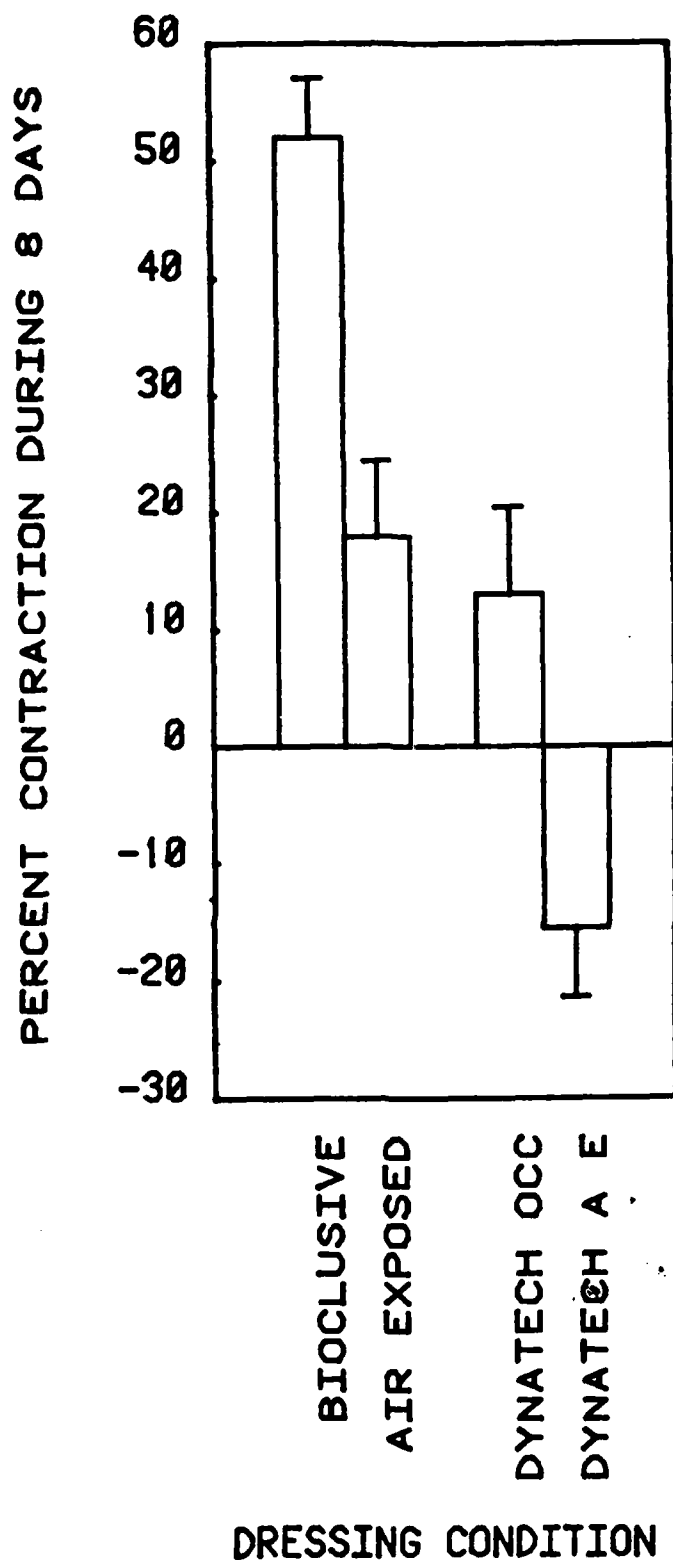


Figure 1. Effect of DYNATECH formed-in-place dressing on the repair of full-thickness excisions on guinea pigs. DYNATECH OCC stands for the DYNATECH dressing covered with BIOCLUSIVE Transparent Film Dressing. A E stands for air exposure.

